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	L #	Hits	Search Text	DBs	Time Stamp
1	L3	14987	biolumines\$ or fluorescen\$ near4 protein\$1 or luciferase\$1 or photoprotein\$1	USPAT; US-PGPUB	2003/02/24 15:09
2	L4	13461 5	bubble\$	USPAT; US-PGPUB	2003/02/24 15:10
3	L5	878	3 and 4	USPAT; US-PGPUB	2003/02/24 15:10
4	L6	15	3 same 4	USPAT; US-PGPUB	2003/02/24 17:01
5	L7	68310	toy or novelty	USPAT; US-PGPUB	2003/02/24 15:40
6	L8	30	5 and 7	USPAT; US-PGPUB	2003/02/24 15:40
7	L9	17	3 same 7	USPAT; US-PGPUB	2003/02/24 17:01
8	L10	20	3 and toy	USPAT; US-PGPUB	2003/02/24 17:09
9	L11	13	10 not 8	USPAT; US-PGPUB	2003/02/24 17:25
10	L12	22	3 and novelty adj item\$1	USPAT; US-PGPUB	2003/02/24 17:24
11	L13	12	12 not 8	USPAT; US-PGPUB	2003/02/24 17:25
12	L14	228	((chemilumines\$ or lumines\$8 or glow\$8) same 4) not 3	USPAT; US-PGPUB	2003/02/24 18:04
13	L15	20	((chemilumines\$ or lumines\$8 or glow\$8) near4 4) not 3	USPAT; US-PGPUB	2003/02/24 17:47
14	L16	6	14 and 7	USPAT; US-PGPUB	2003/02/24 18:04

PGPUB-DOCUMENT-NUMBER: 20030012011

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030012011 A1

TITLE: DECORATIVE ILLUMINATED PUMPKIN STEMS

PUBLICATION-DATE: January 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wyss, John Raymond	Seattle	WA	US	
Latourette, Wade	Portland	OR	US	
Garrett				

APPL-NO: 09/ 905821

DATE FILED: July 16, 2001

US-CL-CURRENT: 362/34,362/122 ,362/806

ABSTRACT:

This invention is in the area of three-dimensional holiday decorations and their manufacture, and specifically in the area of an illuminated decoration which may replace natural stems for the tops of pumpkins, squash and gourd-type fruits as used in Halloween, Thanksgiving, Harvest, Christmas and similar holiday-type decorations. An artificial replacement stem and various assorted appendages are provided. A decorative novelty item, such as a Halloween pumpkin, is affixed with said artificial stem or various appendages of a design or other festive decorative appurtenance, replacing or augmenting its natural stem. Said artificial stem or appendages may also be equipped with means of illumination, especially by chemical luminescence, together with a means for affixing said artificial stem or appendage to said pumpkin or like-type fruit such as adhesive, a spike or hook-type attachment means.

----- KWIC -----

Abstract Paragraph - ABTX:

This invention is in the area of three-dimensional holiday decorations and their manufacture, and specifically in the area of an illuminated decoration which may replace natural stems for the tops of pumpkins, squash and gourd-type fruits as used in Halloween, Thanksgiving, Harvest, Christmas and similar holiday-type decorations. An artificial replacement stem and various assorted appendages are provided. A decorative novelty item, such as a Halloween pumpkin, is affixed with said artificial stem or various appendages of a design

or other festive decorative appurtenance, replacing or augmenting its natural stem. Said artificial stem or appendages may also be equipped with means of illumination, especially by chemical luminescence, together with a means for affixing said artificial stem or appendage to said pumpkin or like-type fruit such as adhesive, a spike or hook-type attachment means.

Summary of Invention Paragraph - BSTX:

[0007] A further object is to provide various adorning appendages to the natural pumpkin, squash or other gourd-type fruit any of which having means for illumination. The above and other objects of the invention are achieved in the following disclosed embodiments by providing an attachable artificial replacement stem or appendage comprising a stem piece or a stem-like body which has the appearance of an actual natural stem, or may take the form of some appendage such as warts or an apparition. The said appendage may be of some type of a decorative holiday theme, an apparition or the like, or jewel-like bubbles, similar to "warts" found on certain gourd-type fruits. Said "jeweled bubbles" or "warts" are made to be easily attachable to the fruit surface, and glow by chemically luminescent means. Said artificial stem piece may be made from a choice of a wide variety of materials, for example, plastic, glass, acrylic or many other translucent, reflective or decorative-type materials.

US-PAT-NO: 6513945

DOCUMENT-IDENTIFIER: US 6513945 B1

TITLE: Decorative illuminated pumpkin stems

DATE-ISSUED: February 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wyss; John Raymond	Seattle	WA	98103	N/A
Latourette; Wade Garrett	Portland	OR	97219	N/A

APPL-NO: 09/ 905821

DATE FILED: July 16, 2001

US-CL-CURRENT: 362/34; 362/122 ; 362/806 ; 362/84

ABSTRACT:

This invention is in the area of three-dimensional holiday decorations and their manufacture, and specifically in the area of an illuminated decoration which may replace natural stems for the tops of pumpkins, squash and gourd-type fruits as used in Halloween, Thanksgiving, Harvest, Christmas and similar holiday-type decorations. An artificial replacement stem and various assorted appendages are provided. A decorative novelty item, such as a Halloween pumpkin, is affixed with said artificial stem or various appendages of a design or other festive decorative appurtenance, replacing or augmenting its natural stem. Said artificial stem or appendages may also be equipped with means of illumination, especially by chemical luminescence, together with a means for affixing said artificial stem or appendage to said pumpkin or like-type fruit such as adhesive, a spike or hook-type attachment means.

3 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

Abstract Text - ABTX:

This invention is in the area of three-dimensional holiday decorations and their manufacture, and specifically in the area of an illuminated decoration which may replace natural stems for the tops of pumpkins, squash and gourd-type

fruits as used in Halloween, Thanksgiving, Harvest, Christmas and similar holiday-type decorations. An artificial replacement stem and various assorted appendages are provided. A decorative **novelty** item, such as a Halloween pumpkin, is affixed with said artificial stem or various appendages of a design or other festive decorative appurtenance, replacing or augmenting its natural stem. Said artificial stem or appendages may also be equipped with means of illumination, especially by chemical luminescence, together with a means for affixing said artificial stem or appendage to said pumpkin or like-type fruit such as adhesive, a spike or hook-type attachment means.

#### Brief Summary Text - BSTX:

A further object is to provide various adorning appendages to the natural pumpkin, squash or other gourd-type fruit any of which having means for illumination. The above and other objects of the invention are achieved in the following disclosed embodiments by providing an attachable artificial replacement stem or appendage comprising a stem piece or a stem-like body which has the appearance of an actual natural stem, or may take the form of some appendage such as warts or an apparition. The said appendage may be of some type of a decorative holiday theme, an apparition or the like, or jewel-like **bubbles**, similar to "warts" found on certain gourd-type fruits. Said "jeweled **bubbles**" or "warts" are made to be easily attachable to the fruit surface, and **glow** by chemically **luminescent** means. Said artificial stem piece may be made from a choice of a wide variety of materials, for example, plastic, glass, acrylic or many other translucent, reflective or decorative-type materials.

US-PAT-NO: 6355593

DOCUMENT-IDENTIFIER: US 6355593 B1

TITLE: Fischer-Tropsch catalyst enhancement

DATE-ISSUED: March 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Daage; Michel A.	Baton Rouge	LA	N/A	N/A
Koveal; Russell John	Baton Rouge	LA	N/A	N/A
Lapidus; Albert	Moscow	N/A	N/A	RU
L'Vovich	Moscow	N/A	N/A	RU
Krylova; Alla Jurievna	Baton Rouge	LA	N/A	N/A
Brennan; Shawn Paul				

APPL-NO: 09/ 654183

DATE FILED: September 1, 2000

US-CL-CURRENT: 502/111; 502/104 ; 502/107 ; 502/326 ; 518/715

ABSTRACT:

A process of enhancing both the activity and the methane selectivity of a Dispersed Active Metal ("DAM") hydrogenation catalyst is disclosed wherein the DAM undergoes low temperature oxidation in a slurry phase to form an oxidized catalyst precursor that is unique in comparison to those formed by conventional high temperature deactivation processes. The oxidized catalyst precursor, which is stable, is subsequently reduced to form an enhanced catalyst by treatment with hydrogen-containing gas at elevated temperature. The process is useful in a wide variety of DAMs formed by art-recognized techniques. The process is equally applicable to the enhanced catalysts formed from the oxidized precursors and their use in hydrogenation reactions.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Detailed Description Text - DETX:

It is understood that various other embodiments and modifications in the practice of the invention will be apparent to, and can be readily made by, those of ordinary skill in the art without departing from the scope and spirit of the invention as described above. Accordingly, it is not intended that the

scope of the claims appended hereto be limited to the exact description set forth above, but rather that the claims be construed as encompassing all of the features of patentable novelty that reside in the present invention, including all the features and embodiments that would be treated as equivalents thereof by those skilled in the art to which the invention pertains. The invention is further described with reference to the following experimental work.

#### Detailed Description Text - DETX:

A suspension of commercial catalyst (Raney.RTM. 2700) consisting of about 30 grams cobalt with water having a cobalt to water ratio of at least 5:1 was in a 500 ml three-neck round bottom flask. Air was vigorously bubbled into the flask to stir the slurry into suspension. The temperature of the slurry was raised to 60.degree. C. during which a slow oxidation of the catalyst occurred. After six hours, the airflow was stopped and a small sample of the catalyst removed and dried to verify the loss of pyrophoricity. If the oxidation had not been sufficient, a strong exotherm would have been observed, accompanied by a spotty orange glow upon filtering in air, indication that further oxidation was required. Upon completion of the oxidation, the oxidized cobalt was filtered. In contrast with Example 1, a slight increase in temperature was observed during filtration in air, indicating that further oxidation was occurring during the filtration in air. Most noticeable was the fact that the exotherm generated by contacting with air is controllable as opposed to the exotherm observed when fresh Raney catalyst is contacted with air. The deactivated catalyst was dried in a vacuum oven at 80.degree. C. for two hours and analyzed as in Example 1. The analysis showed that the oxidized catalyst precursor was composed of cobalt metal, CoO, cobalt hydroxide and Co.sub.3 O.sub.4. The O/Co ratio was 0.9, indicating that the precursor was oxidized to a greater degree than the sample filtered in nitrogen formed in Example 1. Furthermore, the presence of Co.sub.3 O.sub.4 is a clear indication that the oxidation of the cobalt under slurry conditions is different from the direct oxidation with air, which is responsible for the formation of Co.sub.3 O.sub.4. The oxidized catalyst precursor was reduced in a fixed-bed reactor under flowing hydrogen, atmospheric pressure, 375.degree. C. for 2 hours. GHSV>20,000. In the event that the resulting reduced catalyst was not to be utilized directly in a Fischer-Tropsch synthesis, it could be stored in an air-free, inert environment.



US-PAT-NO: 6183604

DOCUMENT-IDENTIFIER: US 6183604 B1

TITLE: Durable and efficient equipment for the production of a combustible and non-pollutant gas from underwater arcs and method therefor

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Santilli; Ruggero Maria	Palm Harbor	FL	N/A	N/A

APPL-NO: 09/ 372277

DATE FILED: August 11, 1999

US-CL-CURRENT: 204/172; 422/186.26 ; 422/186.28

ABSTRACT:

A system for producing a clean burning combustible gas comprising an electrically conductive first electrode and an electrically conductive second electrode. A motor coupled to the first electrode is adapted to move the first electrode with respect to the second electrode to continuously move the arc away from the plasma created by the arc. A water tight container for the electrodes is provided with a quantity of water within the tank sufficient to submerge the electrodes.

11 Claims, 4 Drawing figures

Exemplary Claim Number: 10

Number of Drawing Sheets: 4

----- KWIC -----

Brief Summary Text - BSTX:

The main process in these inventions is essentially the following. The arc is generally produced by a DC power unit, such as a welder, operating at low voltage (25-35 V) and high current (300 A to 3,000 A) depending on available Kwh. The high value of the current brings to incandescence the tip of the carbon electrode in the cathode, with consequential disintegration of the carbon crystal, and release of highly ionized carbon atoms to the arc. Jointly, the arc separates the water into highly ionized atoms of Hydrogen and Oxygen. This causes in the immediate surrounding of the arc a high temperature plasma of about 7,000 F, which is composed by highly ionized H, O and C atoms.

A number of chemical reactions then occur within or near said plasma, such as: the formation of the  $H_2$  and  $O_2$  molecule; the burning of H and O into  $H_2O$ ; the burning of C and O into CO; the burning of CO and O into  $CO_2$ ; and other reactions. Since all these reactions are highly exothermic, they result in the typical, very intense **glow** of the arc within water, which is bigger than that of the same arc in air. The resulting gases cool down in the water surrounding the discharge, and **bubble** to the surface, where they are collected with various means. According to numerous measurements conducted at various independent laboratories, the combustible gas produced with the above process essentially consists of 45%-48%  $H_2$ , 36%-38% CO, 8%-10%  $CO_2$ , and 1%-2%  $O_2$ , the remaining gas consisting of parts per million of more complex molecules composed by H, O and C.

#### Brief Summary Text - BSTX:

These together with other objects of the invention, along with the various features of **novelty** which characterize the invention, are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its operating advantages and the specific objects attained by its uses, reference should be had to the accompanying drawings and descriptive matter in which there is illustrated preferred embodiments of the invention.

US-PAT-NO: 5961894

DOCUMENT-IDENTIFIER: US 5961894 A

TITLE: Black light bubbles

DATE-ISSUED: October 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Swetland, Jr.; Wallace	Gaithersburg	MD	20878	N/A
Byron	Gaithersburg	MD	20878	N/A
Swetland; Melody Sue				

APPL-NO: 09/ 105260

DATE FILED: June 26, 1998

US-CL-CURRENT: 252/700; 252/301.16 ; 252/301.33 ; 252/587 ; 252/588 ; 252/589

ABSTRACT:

Bubbles are formed for entertainment and decorative purposes utilizing a liquid solution that includes a sufficient amount of a surface active agent to form the bubbles and a sufficient amount of a fluorescent agent to provide illumination of the bubbles when viewed in the dark and under an external source of invisible ultraviolet or infrared radiation, such as a black light. The bubble solution is a pre-mixed, non-toxic solution that includes the surface active agent, or soap, to provide formation of the bubble and a fluorescent agent such as Radiant.RTM. fluorescent pigment dispersions to react to the illumination under the external source of invisible ultraviolet or infrared radiation such as a black light. Alternatively, the solution may be spread on a surface in decorative designs to be illuminated with the black light.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Other methods of forcing air through an article to produce bubbles have been developed. One example involves shaping the article as a child's toy and requiring the operator to move the toy in a particular fashion to produce the bubbles. One such toy is a child's plastic toy lawn mower where the bubble solution is poured into an opening that houses the bubble solution and a fan

apparatus and when the child pushes the mower and makes the wheels turn, the fan apparatus inside the mower turns, generating air and producing bubbles.

Brief Summary Text - BSTX:

One example of a limited approach to creating special-effects bubbles is U.S. Pat. No. 5,246,631 Self-Illuminated Bubbles. This patent embodies a combination of a chemiluminescent agent with a bubble solution to produce bubbles that glow in the dark. Self-Illuminated Bubbles do not, however, illuminate under a black light or other external ultraviolet or infrared radiation source. An additional significant limitation of the Self-Illuminated Bubbles is that the product is delivered in component parts that manually must be prepared prior to blowing the bubbles. The bubble solution must be mixed with the chemiluminescent agent by the consumer immediately prior to use. Requiring the consumer to mix the product is time-consuming and messy. Moreover, young children would need the assistance of an older person to mix the solution before using it.

Brief Summary Text - BSTX:

Two more examples of special effects bubbles, by the same inventor, are U.S. Pat. No. 4,284,534, Aqueous Bubble Blowing Composition, and U.S. Pat. No. 4,511,497. Bubble Composition Using Multipurpose Surfactant Base (hereinafter collectively referred to as "Bubble Compositions"). Bubble Compositions are chemical patents that describe bubble solutions capable of accepting many different additives to produce various special effects, such as long-distance flying bubbles that withstand wind turbulence and evade collision; a stream of 80 to 120 floating bubbles; bubbles that burst with a crackle noise; and colored bubbles that turn into flakes. Bubble Compositions do not embody bubbles that glow under dark conditions. Specifically, Bubble Compositions do not describe bubble solutions that glow under an external ultraviolet or infrared radiation source.

Brief Summary Text - BSTX:

While the field of using bubble solutions for entertainment and recreational purposes is saturated with products that are used to form the bubbles from a basic soapy bubble solution, little progress has been made in the area of modifying the bubble solution itself, rather than the bubble blowing product, to produce a varied form of bubble entertainment. Of the few inventions that have modified the bubble solution itself to create special effects bubbles, none contemplate bubbles that will glow in the dark under an external ultraviolet or infrared radiation source, such as a black light.

Brief Summary Text - BSTX:

The current invention, the black light bubbles solution, is directed to a method of forming bubbles that glow in various colors when viewed in the dark

and under an ultraviolet or infrared radiation source, such as a black light. The black light **bubbles** solution achieves its **glowing** effect through fluorescence. The invention comprises a **bubble** solution combined with a fluorescent agent to provide illumination of the **bubbles** when viewed under an external source of invisible ultraviolet or infrared radiation. Fluorescence is defined as "the emission of electromagnetic radiation, especially of visible light, resulting from the absorption of incident radiation and persisting only as long as the stimulating radiation is continued." AMERICAN HERITAGE DICTIONARY, 2nd Edition (1985). In this invention, an external source of invisible ultraviolet or infrared radiation, such as a black light, serves as the incident radiation or stimulating radiation. Consequently, this invention embodies a chemical reaction between the **bubble** solution containing a fluorescent agent with an external radiation source to achieve the **glowing** effect; or through fluorescence.

#### Brief Summary Text - BSTX:

One example of a limited special-effects **bubble** solution is U.S. Pat. No. 5,246,631, Self-Illuminated **Bubbles**. This patent embodies a combination of a **chemiluminescent** agent with a **bubble** solution to produce **bubbles that glow** in the dark. Self-Illuminated **Bubbles** do not, however, illuminate under a black light or other external ultraviolet or infrared radiation source. Self-Illuminated **Bubbles** depends on a chemical reaction taking place within the **bubble** solution itself, after the consumer has mixed together the components parts. to create **bubbles that glow** in the dark. This chemical reaction takes place between a **chemiluminescent** agent and the **bubble** solution through **chemiluminescence**. **Chemiluminescence** is defined as, "the emission of light as a result of a chemical reaction at environmental temperatures." AMERICAN HERITAGE DICTIONARY, 2nd Edition (1985). Self-Illuminated **Bubbles** describes the **chemiluminescent** agent used to achieve the self-**glowing** effect as a combination of various chemicals including an oxalate diester, a peroxide, and a fluorescer. The oxidate is necessary for the **chemiluminescent** reaction; the fluorescer is required for light emission with each type of fluorescer giving off a characteristic color; and the peroxide, or activator, is used to initiate the chemical reaction. No light or color emission is possible without the reactor. Consequently, the invention embodied in Self-Illuminated **Bubbles** requires a chemical reaction that takes place within the **bubble** solution itself to achieve the **glow-in-the-dark** effect; or through **chemiluminescence**.

#### Brief Summary Text - BSTX:

Self-Illuminated **Bubbles** require that the consumer mix the **bubble** solution with the **chemiluminescent** agent immediately prior to use. Requiring the consumer to mix the product is time-consuming and messy. Moreover, young children would need the assistance of an older person to mix the solution before using it. Black Light **Bubbles** is delivered to the consumer pre-mixed and ready for use. The consumer is not required to measure or mix any ingredients.

#### Brief Summary Text - BSTX:

Two more examples of special effects bubbles are U.S. Pat. No. 4,284,534, Aqueous Bubble Blowing Composition, and U.S. Pat. No. 4,511,497, Bubble Composition Using Multipurpose Surfactant Base (hereinafter collectively referred to as "Bubble Compositions"). Bubble Compositions are chemical patents that describe bubble solutions capable of accepting many different additives to produce various special effects, such as long-distance flying bubble that withstand wind turbulence and evade collision; a stream of 80 to 120 floating bubbles; bubbles that burst with a crackle noise; and colored bubbles that turn into flakes. Despite the varied special effects suggested in Bubble Compositions, the patents do not describe a special effect wherein the bubble solution will glow in dark conditions. Moreover, Bubble Compositions do not encompass bubbles that glow under an external source of ultraviolet or infrared radiation.

#### Brief Summary Text - BSTX:

Previous inventors of special effects bubbles have not achieved a combination of bubbles that glow in dark conditions and the use of a black light type of light emission under which to view the bubbles. Moreover, previous inventors who have used fluorescence and black light illumination to enhance their products have not applied the technology to entertainment uses directed toward children and young adults and have not combined the technology with recreational bubble solutions. This invention provides a new and unique method of entertainment, demonstration, and recreation using bubbles and fluorescence.

#### Detailed Description Text - DETX:

The current invention, black light bubble solution, discloses a method of forming bubbles that glow in various colors when viewed in the dark and under an ultraviolet or infrared radiation source, such as a black light. This invention utilizes a bubble solution combined with a non-toxic fluorescent agent to provide illumination of the bubbles when viewed under an external source of invisible ultraviolet or infrared radiation.

#### Detailed Description Text - DETX:

Flourescence is defined as "the emission of electromagnetic radiation, especially of visible light, resulting from the absorption of incident radiation and persisting only as long as the stimulating radiation is continued." AMERICAN HERITAGE DICTIONARY, 2nd Edition (1985). In this invention, an external source of invisible ultraviolet or infrared radiation, such as a black light, serves as the incident radiation or stimulating radiation. Consequently, this invention requires a chemical reaction between the bubble solution containing a flourescent agent with an external radiation source to achieve the glow-in-the-dark effect; or through flourescence.

#### Other Reference Publication - OREF:

Chartrand, Sabra, "Chemical Glow Lights Up Bubbles," New York Times, Apr. 7, 1997, at D4.

US-PAT-NO: 5575553

DOCUMENT-IDENTIFIER: US 5575553 A

TITLE: Container using fiber optic imaging

DATE-ISSUED: November 19, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tipton; Tommy B.	Flatonia	TX	78941	N/A

APPL-NO: 08/ 494002

DATE FILED: June 23, 1995

US-CL-CURRENT: 362/101; 362/154 ; 362/31 ; 362/800 ; 362/812 ; 40/324 ; 40/546

ABSTRACT:

An optically transparent container includes a base, a sidewall extending from the base and having indicia formed as recesses. The recesses are emergent upon an exterior surface of the container and the base includes a sealed compartment. A lighting mechanism housed within the compartment, includes a battery for activating at least one diode disposed beneath a lowermost extremity of the sidewall. A switch mechanism positioned within the compartment includes a touch-sensitive switch for energizing the at least one diode for a predetermined time period when the container is touched. The at least one diode is de-energized when one of the predetermined time period expires and the container is touched subsequently. The at least one light-emitting diode is formed in an orientation which is other than parallel to a bottom surface of the container.

15 Claims, 9 Drawing figures

Exemplary Claim Number: 4

Number of Drawing Sheets: 7

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Brief Summary Text - BSTX:

This invention is directed to novelty items incorporating fiber optic principles and more particularly to a container having means, using fiber optic principles, for illuminating an etched pattern formed in a sidewall of the container.



Detailed Description Text - DETX:

In operation, when the glass is right side up, the light emitted from the LED(s) passes upwardly through the sidewall, and emerges at the sites of engraving. The angled facets of the engraving reflect light outwardly from the glass, thereby creating a very distinctive lighting effect. Such an effect is enhanced by opaque upper panel 21 of the base portion, which prevents light from entering the interior region of the glass and tends to concentrate the light in the sidewall. As light travels through the wall of the container, it exits only where an image is etched or embossed, which causes the image to **glow**. The rest of the glass remains clear except for the rim of the glass where in the light exits. Thus, when a carbonated beverage is poured into the glass, thousands of effervescent **bubbles** also transport light, creating a sparkling effect.

Detailed Description Text - DETX:

Another feature of the invention is a plurality of attachments for connection to the glass. The attachments are suitable for use as **novelty** items which evoke a desired theme or motif. Examples of such attachments are shown in FIG. 5A and 5B.

US-PAT-NO: 6152358  
DOCUMENT-IDENTIFIER: US 6152358 A

TITLE: Bioluminescent novelty items

DATE-ISSUED: November 28, 2000

US-CL-CURRENT: 229/87.19; 435/189 ; 493/955

APPL-NO: 09/ 135988

DATE FILED: August 17, 1998

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS," now U.S. Pat. No. 5,876,995. This application is also a continuation-in-part of U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS". U.S. application Ser. No. 08/757,046 is a continuation-in-part of U.S. application Ser. No. 08/597,274. The subject matter of each of U.S. application Ser. No. 08/597,274 and U.S. application Ser. No. 08/757,046 is herein incorporated in its entirety by reference thereto. The disclosures of each of the above noted applications and provisional application is incorporated herein by reference thereto.

US-PAT-NO: 6113886  
DOCUMENT-IDENTIFIER: US 6113886 A

TITLE: Bioluminescent novelty items

DATE-ISSUED: September 5, 2000

US-CL-CURRENT: 424/49; 424/63 ; 424/64 ; 424/69 ; 424/70.1 ; 424/70.6  
; 424/70.7 ; 424/78.02 ; 424/94.4 ; 435/189 ; 510/119 ; 510/135 ; 510/392  
; 510/481

APPL-NO: 09/ 447208

DATE FILED: November 22, 1999

PARENT-CASE:

RELATED APPLICATIONS This application is a divisional of U.S. application Ser. No. 09/135,988 to Bruce Bryan, filed Aug. 17, 1998, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also continuation-in-part of U.S. application Ser. No. 08/757,046, now U.S. Pat. No. 5,876,995, to Bruce Bryan, filed Nov. 25, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also a continuation-in-part of U.S. application Ser. No. 08/597,274, now allowed, to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS". U.S. Pat. No. 09/135,988 is a continuation-in-part of U.S. application Ser. No. 08/757,046, which is a continuation-in-part of U.S. application Ser. No. 08/597,274. The subject matter of each of U.S. application Ser. Nos. 09/135,988, 08/597,274 and 08/757,046 is herein incorporated in its entirety by reference thereto. This application is also related to provisional application Ser. Nos. 60/079,624 and 60/089,367. The disclosures of each of the above noted applications and provisional applications is incorporated herein by reference thereto.

US-PAT-NO: 5876995  
DOCUMENT-IDENTIFIER: US 5876995 A

TITLE: Bioluminescent novelty items

DATE-ISSUED: March 2, 1999

US-CL-CURRENT: 435/189; 426/104 ; 426/250 ; 426/262 ; 426/268 ; 426/383  
; 426/422 ; 426/540 ; 426/590 ; 426/592 ; 426/656 ; 426/66 ; 530/350

APPL-NO: 08/ 757046

DATE FILED: November 25, 1996

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS". The subject matter of U.S. application Ser. No. 08/597,274 is herein incorporated in its entirety by reference thereto.

US-PAT-NO: 4848743

DOCUMENT-IDENTIFIER: US 4848743 A

TITLE: Popping sound toy

DATE-ISSUED: July 18, 1989

US-CL-CURRENT: 473/414; 273/444 ; 446/181

APPL-NO: 07/ 129298

DATE FILED: December 7, 1987

PGPUB-DOCUMENT-NUMBER: 20030013103

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030013103 A1

TITLE: Apparatus and method for detecting and identifying infectious agents

PUBLICATION-DATE: January 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bryan, Bruce J.	Beverly Hills	CA	US	
Gaalema, Stephen	Colorado Springs	CO	US	
Murphy, Randall B.	Irvington	NY	US	

APPL-NO: 10/ 126139

DATE FILED: April 19, 2002

RELATED-US-APPL-DATA:

child 10126139 A1 20020419 parent division-of 08990103 19971212 US GRANTED  
parent-patent 6458547 US non-provisional-of-provisional 60037675 19970211 US  
non-provisional-of-provisional 60033745 19961212 US

US-CL-CURRENT: 435/6,356/319 ,435/287.2 ,435/7.9

ABSTRACT:

Solid phase methods for the identification of an analyte in a biological medium, such as a body fluid, using bioluminescence are provided. A chip designed for performing the method and detecting the bioluminescence is also provided. Methods employing biomineralization for depositing silicon on a matrix support are also provided. A synthetic synapse is also provided.

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional application Serial No. 60/037,675, filed Feb. 11, 1997 and to U.S. Provisional application Serial No. 60/033,745, filed Dec. 12, 1996.

[0002] Certain subject matter in this application is related to subject matter in U.S. application Ser. No. 08/757,046, filed Nov. 25, 1996, to Bruce Bryan entitled "BIOLUMINESCENT NOVELTY ITEMS" (B), and to U.S. application Ser. No. 08/597,274, filed Feb. 6, 1996, to Bruce Bryan, entitled "BIOLUMINESCENT NOVELTY ITEMS". This application is also related to U.S. application Ser. No. 08/908,909, filed Aug. 8, 1997, to Bruce Bryan entitled "DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES" and to U.S. Provisional application Serial No. 60/023,374, filed Aug. 8, 1996, entitled "DETECTION AND

VISUALIZATION OF NEOPLASMS AND OTHER TISSUES", and also to published International PCT application No. WO 97/.,.

[0003] The subject matter of each of the above noted U.S. applications, provisional applications and International application is herein incorporated by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0297] GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with novelty items, as described herein. Similarly, blue fluorescent proteins (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue fluorescent protein from a yellow-emitting luminous bacterium," *Photochem. Photobiol.* 55(2):293-299 (1992); Lee, et al., "Purification of a blue-fluorescent protein from the bioluminescent bacterium *Photobacterium phosphoreum*" *Methods Enzymol.* (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue fluorescence protein from bacterial luciferase" *Biochem. Biophys. Res. Commun.* 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such fluorescent proteins may be used in the methods provided herein for the detection of infectious agents by binding an analyte to one or more anti ligand-GFP conjugate(s) at a plurality of locations and illuminating the chip with light of an appropriate wavelength to cause the fluorescent proteins to fluoresce whereby the emitted fluorescence is detected by the photodiodes in the chip.

PGPUB-DOCUMENT-NUMBER: 20020132318

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020132318 A1

TITLE: Fluorescent proteins

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zhao, Ming	San Diego	CA	US	
Xu, Mingxu	La Jolla	CA	US	
Jiang, Ping	San Diego	CA	US	
Yang, Meng	San Diego	CA	US	

APPL-NO: 10/ 060857

DATE FILED: January 29, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60264932 20010129 US

US-CL-CURRENT: 435/183,435/320.1 ,435/325 ,435/69.1 ,530/350 ,536/23.2

ABSTRACT:

Improved forms of fluorescent protein with high fluorescence and low toxicity are disclosed.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn. 119(e) from provisional application 60/264,932 filed Jan. 29, 2001. The contents of this application are incorporated herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0004] The above documents, each of which is incorporated herein by reference in its entirety, demonstrate that variations in the amino acid sequence of a protein which exhibits fluorescence upon excitation with radiation of shorter wavelength than the fluorescent wavelength provide a range of color choice and intensity. The **fluorescent proteins** have found wide use both in scientific research and in the production of **novelty items**, such as toys. Because the



only requirements for fluorescence are irradiation with a suitable wavelength and because the fluorescence is visible to the naked eye, these proteins have proved convenient markers and have inspired whimsical applications.

PGPUB-DOCUMENT-NUMBER: 20020004942

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020004942 A1

TITLE: Bioluminescent novelty items

PUBLICATION-DATE: January 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bryan, Bruce	Beverly Hills	CA	US	

APPL-NO: 09/ 803211

DATE FILED: March 8, 2001

RELATED-US-APPL-DATA:

child 09803211 A1 20010308 parent continuation-of 09444762 19991122 US PENDING  
child 09444762 19991122 US parent continuation-of 09135988 19980817 US GRANTED  
parent-patent 6152358 US child 09444762 19991122 US parent continuation-of  
08757046 19961125 US GRANTED parent-patent 5876995 US child 09444762 19991122  
US parent continuation-of 08597274 19960206 US GRANTED parent-patent 6247995 US  
non-provisional-of-provisional 60079624 19980327 US  
non-provisional-of-provisional 60089367 19980615 US

US-CL-CURRENT: 800/288

ABSTRACT:

Novelty items that are combinations of articles of manufacture with fluorescent proteins are provided. These novelty items, include combinations of transgenic plants that express a luciferase or a luciferin with plant food that contains a luciferase and a luciferin.

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/444,762 to Bruce Bryan, filed Nov. 22, 1999, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also continuation of U.S. application Ser. No. 09/135,988 to Bruce Bryan, filed Aug. 17, 1998, now U.S. Pat. No. 6,152,358, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also continuation-in-part of U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, now U.S. Pat. No. 5,876,995, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also a continuation-in-part of U.S. application Ser. No. 08/597,274, now allowed, to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS".  
[0002] U.S. application Ser. No. 09/444,762 is a continuation of U.S.

application Ser. No. 09/135,988, which is a continuation-in-part of U.S. application Ser. No. 08/757,046, which is a continuation-in-part of U.S. application Ser. No. 08/597,274. The subject matter of each of U.S. application Ser. Nos. 09/135,988, 08/597,274 and 08/757,046 is herein incorporated in its entirety by reference thereto. This application is also related to provisional application Ser. Nos. 60/079,624 and 60/089,367. The disclosures of each of the above noted patents, applications and provisional applications is incorporated herein by reference thereto.

----- KWIC -----

#### Abstract Paragraph - ABTX:

Novelty items that are combinations of articles of manufacture with fluorescent proteins are provided. These novelty items, include combinations of transgenic plants that express a luciferase or a luciferin with plant food that contains a luciferase and a luciferin.

#### Detail Description Paragraph - DETX:

[0318] Fluorescent proteins (FPs), particularly green fluorescent proteins (GFPs), such as those from *Aequorea* and *Renilla*, and other related proteins can be used in combination with any of the novelty items provided herein, including toys, beverages, foods, cosmetics, paper products and others. The FPs may be used alone with these items or may be added to bioluminescence generating systems or items with such systems as a means of altering the color of the items. Mutein GFPs from *Aequorea* are also known (see, e.g., U.S. Pat. No. 5,625,048).

#### Detail Description Paragraph - DETX:

[0323] GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with novelty items, as described herein. Similarly, blue fluorescent proteins (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue fluorescent protein from a yellow-emitting luminous bacterium," *Photochem. Photobiol.* 55(2):293-299 (1992); Lee, et al., "Purification of a blue-fluorescent protein from the bioluminescent bacterium *Photobacterium phosphoreum*" *Methods Enzymol. (Biolumin. Chemilumin.)* 57:226-234 (1978); and Gast, et al. "Separation of a blue fluorescence protein from bacterial luciferase" *Biochem. Biophys. Res. Commun.* 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such fluorescent proteins may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the fluorescent proteins to fluoresce.

Detail Description Paragraph - DETX:

[0324] GFPs and/or BFPs or other such **fluorescent proteins** may be used in any of the **novelty items** and combinations provided herein, such as the beverages and toys, including bubble making toys, particularly bubble-making compositions or mixtures and cosmetics. Such systems are particularly of interest because no luciferase is needed to activate the photoprotein and because the proteins are readily digested. These **fluorescent proteins** may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Detail Description Paragraph - DETX:

[0331] As described above for GFPs & BFPs, phycobiliproteins are also activated by visible light of the appropriate wavelength and thus may be used in the absence of luciferase and in conjunction with an external light source to illuminate **novelty items**, particularly, as described herein. In particular, phycobiliproteins may be used in the **novelty items**, such as beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce. Cosmetics containing these proteins are also contemplated.

Detail Description Paragraph - DETX:

[0529] Kits containing such soap compositions, with preferably a moderate pH [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin and the **fluorescent protein** are provided herein. These kits, for example, can be used with a bubble-blowing or producing **toy**. These kits can also include a reloading or charging cartridge, such as the cartridges provided herein.

US-PAT-NO: 6458547

DOCUMENT-IDENTIFIER: US 6458547 B1

TITLE: Apparatus and method for detecting and identifying infectious agents

DATE-ISSUED: October 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan; Bruce J.	Beverly Hills	CA	N/A	N/A
Gaalema; Stephen	Colorado Springs	CO	N/A	N/A
Murphy; Randall B.	Irvington	NY	N/A	N/A

APPL-NO: 08/ 990103

DATE FILED: December 12, 1997

PARENT-CASE:

RELATED APPLICATIONS This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional application Ser. No. 60/037,675, filed Feb. 11, 1997 and to U.S. Provisional application Ser. No. 60/033,745, filed Dec. 12, 1996.

US-CL-CURRENT: 435/7.1; 356/215 ; 356/222 ; 356/317 ; 422/57 ; 422/58 ; 422/82.05 ; 422/82.08 ; 435/288.7 ; 435/6 ; 435/808 ; 435/973 ; 435/975 ; 436/172 ; 436/527 ; 436/805

ABSTRACT:

Solid phase methods for the identification of an analyte in a biological medium, such as a body fluid, using bioluminescence are provided. A chip designed for performing the method and detecting the bioluminescence is also provided. Methods employing biomineralization for depositing silicon on a matrix support are also provided. A synthetic synapse is also provided.

66 Claims, 20 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

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Detailed Description Text - DETX:

GFPs are activated by blue light to emit green light and thus may be used in

the absence of luciferase and in conjunction with an external light source with **novelty items**, as described herein. Similarly, blue **fluorescent proteins** (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue **fluorescent protein** from a yellow-emitting luminous bacterium," *Photochem. Photobiol.* 55(2):293-299 (1992); Lee, et al., "Purification of a blue-**fluorescent protein** from the bioluminescent bacterium *Photobacterium phosphoreum*" *Methods Enzymol.* (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue **fluorescence protein** from bacterial luciferase" *Biochem. Biophys. Res. Commun.* 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such **fluorescent proteins** may be used in the methods provided herein for the detection of infectious agents by binding an analyte to one or more anti ligand-**GFP** conjugate(s) at a plurality of locations and illuminating the chip with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce whereby the emitted fluorescence is detected by the photodiodes in the chip.

US-PAT-NO: 6436682

DOCUMENT-IDENTIFIER: US 6436682 B1

TITLE: Luciferases, fluorescent proteins, nucleic acids encoding the luciferases and fluorescent proteins and the use thereof in diagnostics, high throughput screening and novelty items

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan; Bruce J.	Beverly Hills	CA	N/A	N/A
Szent-Gyorgyi; Christopher	Pittsburgh	PA	N/A	N/A

APPL-NO: 09/ 609161

DATE FILED: June 30, 2000

PARENT-CASE:

RELATED APPLICATIONS This application is a divisional of U.S. application Ser. No. 09/277,716, filed Mar. 26, 1999 to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "LUCIFERASES, FLUORESCENT PROTEINS, NUCLEIC ACIDS ENCODING THE LUCIFERASES AND FLUORESCENT PROTEINS AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND NOVELTY ITEMS." Now U.S. Pat. No. 6,232,107, filed May 15, 2001. This application also claims priority to U.S. provisional application Ser. No. 60/102,939, filed Oct. 1, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "LUCIFERASES, FLUORESCENT PROTEINS, NUCLEIC ACIDS ENCODING THE LUCIFERASES AND FLUORESCENT PROTEINS AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND NOVELTY ITEMS".

Priority is also claimed to U.S. provisional application Serial No. 60/089,367, filed Jun. 15, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "GAUSSIA LUCIFERASE, NUCLEIC ACIDS ENCODING THE LUCIFERASE AND METHODS USING THE LUCIFERASE", and to U.S. provisional application Serial No. 60/079,624, filed Mar. 27, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "RENILLA GREEN FLUORESCENT PROTEIN COMPOSITIONS AND METHODS." Benefit

of priority to each of these applications is claimed under 35 U.S.C. .sctn.119(e). This application is also related to subject matter in U.S. application Ser. No. 08/757,046, filed Nov. 25, 1996, to Bruce Bryan entitled "BIOLUMINESCENT NOVELTY ITEMS", now U.S. Pat. No. 5,876,995, issued Mar. 2, 1999, and in U.S. application Ser. No. 08/597,274, filed Feb. 6, 1996, to Bruce Bryan, entitled "BIOLUMINESCENT NOVELTY ITEMS". This application is also

related to U.S. application Ser. No. 08/908,909, filed Aug. 8, 1997, to Bruce Bryan entitled "DETECTION AND VISUALIZATION OF NEOPLASTIC TISSUE AND OTHER TISSUES". The application is also related to U.S. application Ser. No. 08/990,103, filed Dec. 12, 1997, to Bruce Bryan entitled "APPARATUS AND METHODS FOR DETECTING AND IDENTIFYING INFECTIOUS AGENTS". The subject matter of each of the above noted U.S. applications and provisional applications is herein incorporated by reference in its entirety.

US-CL-CURRENT: 435/189; 124/74 ; 124/76 ; 222/1 ; 42/54 ; 435/183 ; 446/473

ABSTRACT:

Isolated and purified nucleic acid molecules that encode a luciferase from Renilla mulleri, Gaussia and Pleuromamma, and the proteins encoded thereby are provided. Isolated and purified nucleic acids encoding green fluorescent proteins from the genus Renilla and Ptilosarcus, and the green fluorescent proteins encoded thereby are also provided. Compositions and combinations comprising the green fluorescent proteins and/or the luciferase are further provided.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

----- KWIC -----

TITLE - TI:

Luciferases, fluorescent proteins, nucleic acids encoding the luciferases and fluorescent proteins and the use thereof in diagnostics, high throughput screening and novelty items

Parent Case Text - PCTX:

This application is a divisional of U.S. application Ser. No. 09/277,716, filed Mar. 26, 1999 to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "LUCIFERASES, FLUORESCENT PROTEINS, NUCLEIC ACIDS ENCODING THE LUCIFERASES AND FLUORESCENT PROTEINS AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND NOVELTY ITEMS." Now U.S. Pat. No. 6,232,107, filed May 15, 2001.

Parent Case Text - PCTX:

This application also claims priority to U.S. provisional application Ser.



No. 60/102,939, filed Oct. 1, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "LUCIFERASES, FLUORESCENT PROTEINS, NUCLEIC ACIDS ENCODING THE LUCIFERASES AND FLUORESCENT PROTEINS AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND NOVELTY ITEMS". Priority is also claimed to U.S. provisional application Serial No. 60/089,367, filed Jun. 15, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "GAUSSIA LUCIFERASE, NUCLEIC ACIDS ENCODING THE LUCIFERASE AND METHODS USING THE LUCIFERASE", and to U.S. provisional application Serial No. 60/079,624, filed Mar. 27, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "RENILLA GREEN FLUORESCENT PROTEIN COMPOSITIONS AND METHODS." Benefit of priority to each of these applications is claimed under 35 U.S.C. .sctn.119(e).

Brief Summary Text - BSTX:

Recombinant cells containing heterologous nucleic acid encoding a Gaussia luciferase are also provided. Purified Gaussia luciferases and compositions containing a Gaussia luciferase alone or in combination with at other components of a bioluminescence-generating system, such as a Renilla green fluorescent protein, are provided. The Gaussia luciferase can be used, for example, to provide fluorescent illumination of novelty items or used in methods of detecting and visualizing neoplastic tissue and other tissues, detecting infectious agents using immunoassays, such homogenous immunoassays and in vitro fluorescent-based screening assays using multi-well assay devices, or provided in kits for carrying out any of the above-described methods. In particular, the Gaussia luciferase may be used in conjunction with suitable fluorescent proteins in assays provided herein.

Brief Summary Text - BSTX:

Recombinant cells containing heterologous nucleic acid encoding a Ptilosarcus GFP, Renilla GFP, Renilla mulleri luciferase, Gaussia luciferase, and Pleuromamma luciferase are also provided. Purified Renilla mulleri GFP, Renilla reniformis GFP peptides and compositions containing a Renilla GFPs and GFP peptides alone or in combination with at least one component of a bioluminescence-generating system, such as a Renilla mulleri luciferase, are provided. The Renilla GFP and GFP peptide compositions can be used, for example, to provide fluorescent illumination of novelty items or used in methods of detecting and visualizing neoplastic tissue and other tissues, detecting infectious agents using immunoassays, such homogenous immunoassays and in vitro fluorescent-based screening assays using multi-well assay devices, or provided in kits for carrying out any of the above-described methods. In particular, these proteins may be used in FP [fluorescence polarization] assays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays and HTRF [homogeneous time-resolved fluorescence] assays and also in the BRET assays and sensors provided herein.

Brief Summary Text - BSTX:

Compositions containing a Renilla or Ptilosarcus GFP are provided. The compositions can take any of a number of forms, depending on the intended method of use therefor. In certain embodiments, for example, the compositions contain a Renilla GFP or GFP peptide, preferably Renilla mulleri GFP or Renilla reniformis GFP peptide, formulated for use in luminescent novelty items, immunoassays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays, HTRF [homogeneous time-resolved fluorescence] assays or used in conjunction with multi-well assay devices containing integrated photodetectors, such as those described herein. In other instances, the GFPs are used in beverages, foods or cosmetics.

#### Brief Summary Text - BSTX:

Combinations containing a first composition containing a Renilla mulleri GFP or Ptilosarcus GFP or mixtures thereof and a second composition containing a bioluminescence-generating system for use with inanimate articles of manufacture to produce novelty items are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include, but are not limited to: toys, particularly squirt guns, toy cigarettes, toy "Halloween" eggs, footbags and board/card games; finger paints and other paints, slimy play material; textiles, particularly clothing, such as shirts, hats and sports gear suits, threads and yarns; bubbles in bubble making toys and other toys that produce bubbles; balloons; figurines; personal items, such as bath powders, body lotions, gels, powders and creams, nail polishes, cosmetics including make-up, toothpastes and other dentifrices, soaps, cosmetics, body paints, and bubble bath, bubbles made from non-detergent sources, particularly proteins such as albumin and other non-toxic proteins; in fishing lures, particularly cross-linked polyacrylamide containing a fluorescent protein and/or components of a bioluminescence generating system, which glow upon contact with water; items such as inks, paper; foods, such as gelatins, icings and frostings; fish food containing luciferins and transgenic fish, particularly transgenic fish that express a luciferase; plant food containing a luciferin or luciferase, preferably a luciferin for use with transgenic plants that express luciferase; and beverages, such as beer, wine, champagne, soft drinks, and ice cubes and ice in other configurations; fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable form.

#### Brief Summary Text - BSTX:

Kits containing the GFPs for use in the methods, including those described herein, are provided. In one embodiment, the kits containing an article of manufacture and appropriate reagents for generating bioluminescence are provided. The kits containing such soap compositions, with preferably a moderate pH [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin and the GFP are provided herein. These kits, for example, can be used with a bubble-blowing or producing toy. These kits can also include a reloading or charging cartridge or can be used in connection with a food.

Detailed Description Text - DETX:

GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with novelty items, as described herein. Similarly, blue fluorescent proteins (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue fluorescent protein from a yellow-emitting luminous bacterium," Photochem. Photobiol. 55(2):293-299 (1992); Lee, et al., "Purification of a blue-fluorescent protein from the bioluminescent bacterium *Photobacterium phosphoreum*" Methods Enzymol. (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue fluorescence protein from bacterial luciferase" Biochem. Biophys. Res. Commun. 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such fluorescent proteins may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the fluorescent proteins to fluoresce.

Detailed Description Text - DETX:

GFPs and/or BFPs or other such fluorescent proteins may be used in any of the novelty items and combinations provided herein, such as the beverages and toys, including bubble making toys, particularly bubble-making compositions or mixtures. Also of particular interest are the use of these proteins in cosmetics, particularly face paints or make-up, hair colorants or hair conditioners, mousses or other such products. Such systems are particularly of interest because no luciferase is needed to activate the photoprotein and because the proteins are non-toxic and safe to apply to the skin, hair, eyes and to ingest. These fluorescent proteins may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Detailed Description Text - DETX:

Compositions containing a Gaussia luciferase are provided. The compositions may also contain a Renilla GFP or GFP peptide. The compositions can take any of a number of forms, depending on the intended method of use therefor. In certain embodiments, the compositions are prepared for use in bioluminescent novelty items, immunoassays or FRET and FET assays. The compositions may also be used in conjunction with multi-well assay devices containing integrated photodetectors (see, e.g., copending U.S. application Ser. No. 08/990,103), for detection of tumors (see, e.g., U.S. application Ser. No. 08/908,909), or in bioluminescent novelty items (see, U.S. application Ser. Nos. 08/597,274 and 08/757,046).

Detailed Description Text - DETX:

Compositions containing a Renilla GFP or GFP peptide are provided. The compositions can take any of a number of forms, depending on the intended method of use therefor. In certain embodiments, for example, the compositions contain a Renilla GFP or GFP peptide, preferably Renilla muller GFP or Renilla reniformis GFP peptide, formulated for use in luminescent novelty items, immunoassays, FRET and FET assays. The compositions may also be used in conjunction with multi-well assay devices containing integrated photodetectors, such as those described herein.

US-PAT-NO: 6232107

DOCUMENT-IDENTIFIER: US 6232107 B1

TITLE: Luciferases, fluorescent proteins, nucleic acids encoding the luciferases and fluorescent proteins and the use thereof in diagnostics, high throughput screening and novelty items

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan; Bruce J.	Beverly Hills	CA	90210	N/A
Szent-Gyorgyi; Christopher	Pittsburgh	PA	N/A	N/A

APPL-NO: 09/ 277716

DATE FILED: March 26, 1999

PARENT-CASE:

RELATED APPLICATIONS This application claims priority to U.S. provisional application Ser. No. 60/102,939, filed Oct. 1, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "LUCIFERASES, FLUORESCENT PROTEINS, NUCLEIC ACIDS ENCODING THE LUCIFERASES AND FLUORESCENT PROTEINS AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND NOVELTY ITEMS". Priority is also claimed to U.S. provisional application Ser. No.60/089,367, filed Jun. 15, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "GAUSSIA LUCIFERASE, NUCLEIC ACIDS ENCODING THE LUCIFERASE AND METHODS USING THE LUCIFERASE", and to U.S. provisional application Ser. No.60/079,624, filed Mar. 27, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "RENILLA GREEN FLUORESCENT PROTEIN COMPOSITIONS AND METHODS." For U.S. purposes, benefit of priority to each of these applications is claimed under 35 U.S.C. .sctn.119(e). This application is also related to subject matter in U.S. application Ser. No. 08/757,046, filed Nov. 25, 1996, to Bruce Bryan entitled "BIOLUMINESCENT NOVELTY ITEMS", now U.S. Pat. No. 5,876,995, issued Mar. 2, 1999, and in U.S. application Ser. No. 08/597,274, filed Feb. 6, 1996, to Bruce Bryan, entitled "BIOLUMINESCENT NOVELTY ITEMS". This application is also related to U.S. application Ser. No. 08/908,909, filed Aug. 8, 1997, to Bruce Bryan entitled "DETECTION AND VISUALIZATION OF NEOPLASTIC TISSUE AND OTHER TISSUES". The application is also related to U.S. application Ser. No. 08/990,103, filed Dec. 12, 1997, to Bruce Bryan entitled "APPARATUS AND METHODS FOR DETECTING AND IDENTIFYING INFECTIOUS AGENTS". The subject matter of each of the above noted U.S. applications and provisional applications is herein incorporated by reference in its entirety.

US-CL-CURRENT: 435/189; 435/183 ; 435/252.2 ; 435/320.1 ; 435/6 ; 435/69.1 ; 435/8

ABSTRACT:

Isolated and purified nucleic acid molecules that encode a luciferase from Renilla mulleri, Gaussia and Pleuromamma, and the proteins encoded thereby are provided. Isolated and purified nucleic acids encoding green fluorescent proteins from the genus Renilla and Ptilosarcus, and the green fluorescent proteins encoded thereby are also provided. Compositions and combinations comprising the green fluorescent proteins and/or the luciferase are further provided.

63 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

----- KWIC -----

TITLE - TI:

Luciferases, **fluorescent proteins**, nucleic acids encoding the luciferases and **fluorescent proteins** and the use thereof in diagnostics, high throughput screening and **novelty items**

Parent Case Text - PCTX:

This application claims priority to U.S. provisional application Ser. No. 60/102,939, filed Oct. 1, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "LUCIFERASES, **FLUORESCENT PROTEINS**, NUCLEIC ACIDS ENCODING THE LUCIFERASES AND **FLUORESCENT PROTEINS** AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND **NOVELTY ITEMS**". Priority is also claimed to U.S. provisional application Ser. No. 60/089,367, filed Jun. 15, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "GAUSSIA LUCIFERASE, NUCLEIC ACIDS ENCODING THE LUCIFERASE AND METHODS USING THE LUCIFERASE", and to U.S. provisional application Ser. No. 60/079,624, filed Mar. 27, 1998, to Bruce Bryan and Christopher Szent-Gyorgyi, entitled "RENILLA GREEN **FLUORESCENT PROTEIN** COMPOSITIONS AND METHODS." For U.S. purposes, benefit of priority to each of these applications is claimed under 35 U.S.C. .sctn.119(e).

Brief Summary Text - BSTX:

Recombinant cells containing heterologous nucleic acid encoding a Gaussia luciferase are also provided. Purified Gaussia luciferases and compositions containing a Gaussia luciferase alone or in combination with at other components of a bioluminescence-generating system, such as a Renilla green

fluorescent protein, are provided. The Gaussia luciferase can be used, for example, to provide fluorescent illumination of novelty items or used in methods of detecting and visualizing neoplastic tissue and other tissues, detecting infectious agents using immunoassays, such homogenous immunoassays and in vitro fluorescent-based screening assays using multi-well assay devices, or provided in kits for carrying out any of the above-described methods. In particular, the Gaussia luciferase may be used in conjunction with suitable fluorescent proteins in assays provided herein.

#### Brief Summary Text - BSTX:

Recombinant cells containing heterologous nucleic acid encoding a Ptilosarcus GFP, Renilla GFP, Renilla mulleri luciferase, Gaussia luciferase, and Pleuromamma luciferase are also provided. Purified Renilla mulleri GFP, Renilla reniformis GFP peptides and compositions containing a Renilla GFPs and GFP peptides alone or in combination with at least one component of a bioluminescence-generating system, such as a Renilla mulleri luciferase, are provided. The Renilla GFP and GFP peptide compositions can be used, for example, to provide fluorescent illumination of novelty items or used in methods of detecting and visualizing neoplastic tissue and other tissues, detecting infectious agents using immunoassays, such homogenous immunoassays and In vitro fluorescent-based screening assays using multi-well assay devices, or provided in kits for carrying out any of the above-described methods. In particular, these proteins may be used in FP [fluorescence polarization] assays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays and HTRF [homogeneous time-resolved fluorescence] assays and also in the BRET assays and sensors provided herein.

#### Brief Summary Text - BSTX:

Compositions containing a Renilla or Ptilosarcus GFP are provided. The compositions can take any of a number of forms, depending on the intended method of use therefor. In certain embodiments, for example, the compositions contain a Renilla GFP or GFP peptide, preferably Renilla mulleri GFP or Renilla reniformis GFP peptide, formulated for use in luminescent novelty items, immunoassays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays, HTRF [homogeneous time-resolved fluorescence] assays or used in conjunction with multi-well assay devices containing integrated photodetectors, such as those described herein. In other instances, the GFPs are used in beverages, foods or cosmetics.

#### Brief Summary Text - BSTX:

Combinations containing a first composition containing a Renilla mulleri GFP or Ptilosarcus GFP or mixtures thereof and a second composition containing a bioluminescence- generating system for use with inanimate articles of manufacture to produce novelty items are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include, but are not limited to: toys, particularly squirt guns,

toy cigarettes, toy "Halloween" eggs, footbags and board/card games; finger paints and other paints, slimy play material; textiles, particularly clothing, such as shirts, hats and sports gear suits, threads and yarns; bubbles in bubble making toys and other toys that produce bubbles; balloons; figurines; personal items, such as bath powders, body lotions, gels, powders and creams, nail polishes, cosmetics including make-up, toothpastes and other dentifrices, soaps, cosmetics, body paints, and bubble bath, bubbles made from non-detergent sources, particularly proteins such as albumin and other non-toxic proteins; in fishing lures, particularly crosslinked polyacrylamide containing a **fluorescent protein** and/or components of a bioluminescence generating system, which glow upon contact with water; items such as inks, paper; foods, such as gelatins, icings and frostings; fish food containing luciferins and transgenic fish, particularly transgenic fish that express a luciferase; plant food containing a luciferin or luciferase, preferably a luciferin for use with transgenic plants that express luciferase; and beverages, such as beer, wine, champagne, soft drinks, and ice cubes and ice in other configurations; fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable form.

#### Brief Summary Text - BSTX:

Kits containing the GFPs for use in the methods, including those described herein, are provided. In one embodiment, the kits containing an article of manufacture and appropriate reagents for generating bioluminescence are provided. The kits containing such soap compositions, with preferably a moderate Ph [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin and the **GFP** are provided herein. These kits, for example, can be used with a bubble-blowing or producing toy. These kits can also include a reloading or charging cartridge or can be used in connection with a food.

#### Detailed Description Text - DETX:

GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with **novelty items**, as described herein. Similarly, blue **fluorescent proteins** (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al, "A blue **fluorescent protein** from a yellow-emitting luminous bacterium," Photochem. Photobiol. 55(2):293-299 (1992); Lee, et al., "Purification of a blue-**fluorescent protein** from the bioluminescent bacterium *Photobacterium phosphoreum*" Methods Enzymol. (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue **fluorescence protein** from bacterial luciferase" Biochem. Biophys. Res. Commun. 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such **fluorescent proteins** may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the **fluorescent**



proteins to fluoresce.

Detailed Description Text - DETX:

GFPs and/or BFPs or other such fluorescent proteins may be used in any of the novelty items and combinations provided herein, such as the beverages and toys, including bubble making toys, particularly bubble-making compositions or mixtures. Also of particular interest are the use of these proteins in cosmetics, particularly face paints or make-up, hair colorants or hair conditioners, mousses or other such products. Such systems are particularly of interest because no luciferase is needed to activate the photoprotein and because the proteins are non-toxic and safe to apply to the skin, hair, eyes and to ingest. These fluorescent proteins may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Detailed Description Text - DETX:

Compositions containing a Gaussia luciferase are provided. The compositions may also contain a Renilla GFP or GFP peptide. The compositions can take any of a number of forms, depending on the intended method of use therefor. In certain embodiments, the compositions are prepared for use in bioluminescent novelty items, immunoassays or FRET and FET assays. The compositions may also be used in conjunction with multi-well assay devices containing integrated photodetectors (see, e.g., copending U.S. application Ser. No. 08/990,103), for detection of tumors (see, e.g., U.S. application Ser. No. 08/908,909, or in bioluminescent novelty items (see, U.S. application Ser. Nos. 08/597,274 and 08/757,046.

Detailed Description Text - DETX:

Compositions containing a Renilla GFP or GFP peptide are provided. The compositions can take any of a number of forms, depending on the intended method of use therefor. In certain embodiments, for example, the compositions contain a Renilla GFP or GFP peptide, preferably Renilla mulleri GFP or Renilla reniformis GFP peptide, formulated for use in luminescent novelty items, immunoassays, FRET and FET assays. The compositions may also be used in conjunction with multi-well assay devices containing integrated photodetectors, such as those described herein.

US-PAT-NO: 6152358

DOCUMENT-IDENTIFIER: US 6152358 A

TITLE: Bioluminescent novelty items

DATE-ISSUED: November 28, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan; Bruce	Beverly Hills	CA	90210	N/A

APPL-NO: 09/ 135988

DATE FILED: August 17, 1998

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS," now U.S. Pat. No. 5,876,995. This application is also a continuation-in-part of U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS". U.S. application Ser. No. 08/757,046 is a continuation-in-part of U.S. application Ser. No. 08/597,274. The subject matter of each of U.S. application Ser. No. 08/597,274 and U.S. application Ser. No. 08/757,046 is herein incorporated in its entirety by reference thereto. The disclosures of each of the above noted applications and provisional application is incorporated herein by reference thereto.

US-CL-CURRENT: 229/87.19; 435/189 ; 493/955

ABSTRACT:

**Novelty items** that are combinations of articles of manufacture with bioluminescence generating systems and/or **fluorescent proteins** are provided. These **novelty items**, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include toys, paints, slimy play material, textiles, particularly clothing, bubbles in bubble making toys and other toys that produce bubbles, balloons, personal items, such as cosmetics, bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, foods, such as gelatins, icings and frostings, beverages such as beer, wine, champagne, soft drinks, and glowing ice, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable formulation.

58 Claims, 34 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Abstract Text - ABTX:

Novelty items that are combinations of articles of manufacture with bioluminescence generating systems and/or fluorescent proteins are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include toys, paints, slimy play material, textiles, particularly clothing, bubbles in bubble making toys and other toys that produce bubbles, balloons, personal items, such as cosmetics, bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, foods, such as gelatins, icings and frostings, beverages such as beer, wine, champagne, soft drinks, and glowing ice, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable formulation.

Detailed Description Text - DETX:

Fluorescent proteins (FPs), particularly green fluorescent proteins (GFPs), such as those from *Aequorea* and *Renilla*, and other related proteins can be used in combination with any of the novelty items provided herein, including toys, beverages, foods, cosmetics, paper products and others. The FPs may be used alone with these items or may be added to bioluminescence generating systems or items with such systems as a means of altering the color of the items. Mutein GFPs from *Aequorea* are also known (see, e., U.S. Pat. No. 5,625,048).

Detailed Description Text - DETX:

GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with novelty items, as described herein. Similarly, blue fluorescent proteins (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue fluorescent protein from a yellow-emitting luminous bacterium," *Photochem. Photobiol.* 55(2):293-299 (1992); Lee, et al., "Purification of a blue-fluorescent protein from the bioluminescent bacterium *Photobacterium phosphoreum*" *Methods Enzymol.* (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue fluorescence protein from bacterial luciferase" *Biochem. Biophys. Res. Commun.* 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such fluorescent proteins may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the fluorescent

**proteins** to fluoresce.

Detailed Description Text - DETX:

GFPs and/or BFPs or other such **fluorescent proteins** may be used in any of the **novelty items** and combinations provided herein, such as the beverages and toys, including bubble making toys, particularly bubble-making compositions or mixtures and cosmetics. Such systems are particularly of interest because no luciferase is needed to activate the photoprotein and because the proteins are readily digested. These **fluorescent proteins** may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Detailed Description Text - DETX:

As described above for GFPs & BFPs, phycobiliproteins are also activated by visible light of the appropriate wavelength and thus may be used in the absence of luciferase and in conjunction with an external light source to illuminate **novelty items**, particularly, as described herein. In particular, phycobiliproteins may be used in the **novelty items**, such as beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce. Cosmetics containing these proteins are also contemplated.

Detailed Description Text - DETX:

Kits containing such soap compositions, with preferably a moderate pH [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin and the **fluorescent protein** are provided herein. These kits, for example, can be used with a bubble-blowing or producing **toy**. These kits can also include a reloading or charging cartridge, such as the cartridges provided herein.

US-PAT-NO: 6113886

DOCUMENT-IDENTIFIER: US 6113886 A

TITLE: Bioluminescent novelty items

DATE-ISSUED: September 5, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan, Bruce	Beverly Hills	CA	90210	N/A

APPL-NO: 09/ 447208

DATE FILED: November 22, 1999

PARENT-CASE:

RELATED APPLICATIONS This application is a divisional of U.S. application Ser. No. 09/135,988 to Bruce Bryan, filed Aug. 17, 1998, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also continuation-in-part of U.S. application Ser. No. 08/757,046, now U.S. Pat. No. 5,876,995, to Bruce Bryan, filed Nov. 25, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS." This application is also a continuation-in-part of U.S. application Ser. No. 08/597,274, now allowed, to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS". U.S. Pat. No. 09/135,988 is a continuation-in-part of U.S. application Ser. No. 08/757,046, which is a continuation-in-part of U.S. application Ser. No. 08/597,274. The subject matter of each of U.S. application Ser. Nos. 09/135,988, 08/597,274 and 08/757,046 is herein incorporated in its entirety by reference thereto. This application is also related to provisional application Ser. Nos. 60/079,624 and 60/089,367. The disclosures of each of the above noted applications and provisional applications is incorporated herein by reference thereto.

US-CL-CURRENT: 424/49; 424/63 ; 424/64 ; 424/69 ; 424/70.1 ; 424/70.6 ; 424/70.7 ; 424/78.02 ; 424/94.4 ; 435/189 ; 510/119 ; 510/135 ; 510/392 ; 510/481

ABSTRACT:

**Novelty items** that are combinations of articles of manufacture with bioluminescence generating systems and/or **fluorescent proteins** are provided. These **novelty items**, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include toys, personal items, such as cosmetics, bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other formulations.

30 Claims, 34 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Abstract Text - ABTX:

Novelty items that are combinations of articles of manufacture with bioluminescence generating systems and/or fluorescent proteins are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include toys, personal items, such as cosmetics, bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other formulations.

Detailed Description Text - DETX:

Fluorescent proteins (FPs), particularly green fluorescent proteins (GFPs), such as those from *Aequorea* and *Renilla*, and other related proteins can be used in combination with any of the novelty items provided herein, including toys, beverages, foods, cosmetics, paper products and others. The FPs may be used alone with these items or may be added to bioluminescence generating systems or items with such systems as a means of altering the color of the items. Mutein GFPs from *Aequorea* are also known (see, e.g., U.S. Pat. No. 5,625,048).

Detailed Description Text - DETX:

GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with novelty items, as described herein. Similarly, blue fluorescent proteins (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue fluorescent protein from a yellow-emitting luminous bacterium," *Photochem. Photobiol.* 55(2):293-299 (1992); Lee, et al., "Purification of a blue-fluorescent protein from the bioluminescent bacterium *Photobacterium phosphoreum*" *Methods Enzymol.* (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue fluorescence protein from bacterial luciferase" *Biochem. Biophys. Res. Commun.* 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such fluorescent proteins may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the fluorescent

**proteins** to fluoresce.

Detailed Description Text - DETX:

GFPs and/or BFPs or other such **fluorescent proteins** may be used in any of the **novelty items** and combinations provided herein, such as the beverages and toys, including bubble making toys, particularly bubble-making compositions or mixtures and cosmetics. Such systems are particularly of interest because no luciferase is needed to activate the photoprotein and because the proteins are readily digested. These **fluorescent proteins** may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Detailed Description Text - DETX:

As described above for GFPs & BFPs, phycobiliproteins are also activated by visible light of the appropriate wavelength and thus may be used in the absence of luciferase and in conjunction with an external light source to illuminate **novelty items**, particularly, as described herein. In particular, phycobiliproteins may be used in the **novelty items**, such as beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce. Cosmetics containing these proteins are also contemplated.

Detailed Description Text - DETX:

Kits containing such soap compositions, with preferably a moderate pH [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin and the **fluorescent protein** are provided herein. These kits, for example, can be used with a bubble-blowing or producing **toy**. These kits can also include a reloading or charging cartridge, such as the cartridges provided herein.

Claims Text - CLTX:

b) one or more components of a bioluminescence generating system and/or a **fluorescent protein**, whereby the combination is a **novelty item** selected from among personal care items, dentifrices, soaps, body paints and powders, and bubble baths.

US-PAT-NO: 5876995

DOCUMENT-IDENTIFIER: US 5876995 A

TITLE: Bioluminescent novelty items

DATE-ISSUED: March 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan, Bruce	Beverly Hills	CA	90210	N/A

APPL-NO: 08/ 757046

DATE FILED: November 25, 1996

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS". The subject matter of U.S. application Ser. No. 08/597,274 is herein incorporated in its entirety by reference thereto.

US-CL-CURRENT: 435/189; 426/104 ; 426/250 ; 426/262 ; 426/268 ; 426/383 ; 426/422 ; 426/540 ; 426/590 ; 426/592 ; 426/656 ; 426/66 ; 530/350

ABSTRACT:

Systems and apparatus for generating bioluminescence, and combinations of these systems and apparatus with inanimate articles of manufacture to produce novelty items are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, and include toys, paints, slimy play material, textiles, particularly clothing, bubbles in bubble making toys and other toys that produce bubbles, balloons, personal items, such as bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, foods, such as gelatins, icings and frostings, beverages such as beer, wine, champagne, soft drinks, and glowing ice, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable formulation. Cartridges for charging and/or recharging the novelty items with bioluminescence generating systems are also provided.

47 Claims, 34 Drawing figures

Exemplary Claim Number: 25

Number of Drawing Sheets: 9



----- KWIC -----

Detailed Description Text - DETX:

GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source with **novelty items**, as described herein. Similarly, blue **fluorescent proteins** (BFPs), such as from *Vibrio fischeri*, *Vibrio harveyi* or *Photobacterium phosphoreum*, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue **fluorescent protein** from a yellow-emitting luminous bacterium," *Photochem. Photobiol.* 55(2):293-299 (1992); Lee, et al., "Purification of a blue-**fluorescent protein** from the bioluminescent bacterium *Photobacterium phosphoreum*" *Methods Enzymol.* (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue **fluorescence protein** from bacterial luciferase" *Biochem. Biophys. Res. Commun.* 80(1):14-21 (1978), each, as all references cited herein, incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such **fluorescent proteins** may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce.

Detailed Description Text - DETX:

GFPs and/or BFPs or other such **fluorescent proteins** may be used in any of the **novelty items** and combinations provided herein, such as the beverages and toys, including bubble making toys, particularly bubble-making compositions or mixtures. Such systems are particularly of interest because no luciferase is needed to activate the photoprotein and because the proteins are readily digested. These **fluorescent proteins** may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Detailed Description Text - DETX:

As described above for GFPs & BFPs, phycobiliproteins are also activated by visible light of the appropriate wavelength and thus may be used in the absence of luciferase and in conjunction with an external light source to illuminate **novelty items**, particularly, as described herein. In particular, phycobiliproteins may be used in the beverage and/or food combinations provided herein and served in rooms illuminated with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce. As noted above, these proteins may be used in combination with other fluorescent proteins and/or bioluminescence generating systems to produce an array of colors or to provide different colors over time.

Detailed Description Text - DETX:

Kits containing such soap compositions, with preferably a moderate pH [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin and the **fluorescent protein** are provided herein. These kits, for example, can be used with a bubble-blowing or producing **toy**. These kits can also include a reloading or charging cartridge, such as the cartridges provided herein.

Claims Text - CLTX:

an article of manufacture; and a **fluorescent protein**, whereby the combination is a **novelty item**, wherein the article of manufacture is food.

PGPUB-DOCUMENT-NUMBER: 20020160032

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160032 A1

TITLE: Manufacture of bone graft substitutes

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Long, Marc	Memphis	TN	US	
Cooper, Michael B.	Memphis	TN	US	
Kinnane, Keith M.	Bartlett	TN	US	
Allen, Trevor	York	TN	GB	
Schryver, Jeff	Cordova		US	

APPL-NO: 09/ 792681

DATE FILED: February 23, 2001

US-CL-CURRENT: 424/423,264/109

ABSTRACT:

The present invention is directed to methods and compositions for manufacturing a bone graft substitute. A powder compaction process is utilized to generate a shaped product comprised of a granulated bone material, such as demineralized bone matrix. In addition, a processing aid is utilized to facilitate compaction of the granulated bone material and for release of the product from the die.

----- KWIC -----

Detail Description Paragraph - DETX:

[0055] The term "JAX.TM." as used herein is defined as a bone graft substitute particle which generally has the shape of a toy jack. In a specific embodiment, it is a three-dimensional six-armed star shape.

Detail Description Paragraph - DETX:

[0072] In a specific embodiment, the bone material of the present invention is colored to make it more visible. In another specific embodiment, differently shaped BGS of the present invention are denoted with different colors for better differentiation of the particles. In another specific embodiment, the

particles are coated or have contained within them an agent such as green **fluorescent protein** or blue **fluorescent protein** to make them fluorescent and therefore more visible.

PGPUB-DOCUMENT-NUMBER: 20020146807

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146807 A1

TITLE: Novel polypeptides and nucleic acids encoding same

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Li, Li	Cheshire	CT	US	
Prayaga, Sudhirdas K.	O'Fallon	MO	US	
Padigaru, Muralidhara	Branford	CT	US	
MacDougall, John R.	Hamden	CT	US	
Spytek, Kimberly Ann	New Haven	CT	US	
Tchernev, Velizar T.	Branford	CT	US	
Vernet, Corine A. M.	North Branford	CT	US	

APPL-NO: 09/ 771730

DATE FILED: January 29, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60178413 20000127 US  
non-provisional-of-provisional 60178371 20000127 US  
non-provisional-of-provisional 60178408 20000127 US  
non-provisional-of-provisional 60178370 20000127 US  
non-provisional-of-provisional 60178406 20000127 US  
non-provisional-of-provisional 60178414 20000127 US  
non-provisional-of-provisional 60178409 20000127 US  
non-provisional-of-provisional 60180634 20000207 US  
non-provisional-of-provisional 60220516 20000724 US  
non-provisional-of-provisional 60221408 20000728 US  
non-provisional-of-provisional 60221943 20000731 US  
non-provisional-of-provisional 60257599 20001221 US  
non-provisional-of-provisional 60260290 20010108 US

US-CL-CURRENT: 435/252.1,435/7.2 ,530/350

ABSTRACT:

The present invention provides novel isolated NOVX polynucleotides and polypeptides encoded by the NOVX polynucleotides. Also provided are the antibodies that immunospecifically bind to a NOVX polypeptide or any derivative, variant, mutant or fragment of the NOVX polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the NOVX polypeptide, polynucleotide and antibody are utilized in the detection and

treatment of a broad range of pathological states, as well as to other uses.

## RELATED APPLICATIONS

[0001] This application claims priority to, 60/178,413, filed Jan. 27, 2000; 60/178,371, filed Jan. 27, 2000; 60/178,408, filed Jan. 27, 2000; 60/178,370, filed Jan. 27, 2000; 60/178,406, filed Jan. 27, 2000; 60/178,414, filed Jan. 27, 2000; 60/178,409, filed Jan. 27, 2000; 60/180,634, filed Feb. 7, 2000; 60/220,516, filed Jul. 24, 2000; 60/221,408, filed Jul. 28, 2000; 60/221,943, filed Jul. 31, 2000; 60/257,599, filed Dec. 21, 2000; and 60/260,290, filed Jan. 8, 2001, which are incorporated herein by reference in their entirety.

----- KWIC -----

## Detail Description Paragraph - DETX:

[0030] Issel-Tarver and Rine (1996) characterized 4 members of the canine olfactory receptor gene family. The 4 subfamilies comprised genes expressed exclusively in olfactory epithelium. Analysis of large DNA fragments using Southern blots of pulsed field gels indicated that subfamily members were clustered together, and that two of the subfamilies were closely linked in the dog genome. Analysis of the four olfactory receptor gene subfamilies in 26 breeds of dog provided evidence that the number of genes per subfamily was stable in spite of differential selection on the basis of olfactory acuity in scent hounds, sight hounds, and toy breeds.

## Detail Description Table CWU - DETL:

29TABLE 29 ptnr:SPTREMBL-ACC:O70271 OLFACTORY RECEPTOR-LIKE  
PROTEIN-RATTUS  
NORVEGICUS (RAT), 327 aa. Length =327 Plus Strand HSPs: Score =710 (249.9  
bits), Expect =2.7e-69, P =2.7e-69 Identities =133/302 (44%) , Positives  
204/302 (67%) , Frame =1 Query: 70  
ENWTQVTSFVLLGFPSSHLIQFLVFLGLMVTYIVTATGKLLIIVLSW- IDQRLHIQMYFFL 249 N  
T V F +  
**GFP** + + + L FL M + Y + + G + LII + + D RL MYFFL Sbjct: 10  
KNGTLVQEFIEGFPVAEHLRILFFLLHMLAYLASLMGNMLIITY- TCVDHRLQTPMYFFL 69  
Query: 250  
RNFSFLELLLVTVVVPKMLVVILTGDHT- ISFVSCIIQSYLYFFLGTTDFFLLAVMSLDY 429  
FSF + E + T  
V + P + + L + IL + G I F + + C Q + + + FLG FFL + AV + SLDR + Sbjct: 70 STFSFVECCFITTVIPQLLTIIL-  
SGRQKIPFMACFSQAFVVLFLGA AVFFLMAVLSLDRF 129 Query: 430  
LAICRPLRYETLMNGHVCSQLVLASWLAGFLWVLCPTVLMASLPFCGPNIDHFFRDSWP 609  
LAIC + PL Y  
T + M + + C LV S + GFL + + P V + + + + CGPN I HFF D P Sbjct: 130  
LAICKPLEYPTIMSPRMCFLVTVSLVLGFLFMASPVVMLSQSFYCGPNIIPHFFCDFGP 189  
Query: 610  
LLRLSCGDTHLLKLVAFMLSTLVLLGSLALTSVSYACILATV- LRAPTAAERRKAFSTCAS 789 L  
LSC + T  
+ + + + F L + + VL SL + + Y + I + T + + R P + A ER + + AFSTC + S Sbjct: 190

LANLSCSETRSIEMLFFTLAIIVLFTSLLIAIFA- YSTIVVTIVRLPSARERQRAFSTCSS 249

Query: 790

HLTVVVIIYGSSIFLYIRMSEAQSKLLNKGASVLSCIITPLLNPFIPTLRNDKVQQALRE 969 HL V+  
++YGS

+F+Y++ + N+ A +++ ++TPLLNP I+TLRN +V QALR+ Sbjct: 250

HLIVLSLMYGSCVFIYLPKQSRVDTNREAVLVNMVVTPLLNPVIYTLRNKQVHQALRD 309

Query: 970

AL 975 SEQ ID NO:61 AL Sbjct: 310 AL 311 SEQ ID NO:62

PGPUB-DOCUMENT-NUMBER: 20020090659

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020090659 A1

TITLE: Detection and visualization of neoplastic tissues and other tissues

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bryan, Bruce	Beverly Hills	CA	US	

APPL-NO: 09/ 746485

DATE FILED: December 22, 2000

RELATED-US-APPL-DATA:

child 09746485 A1 20001222 parent continuation-of 08908909 19970808 US UNKNOWN

US-CL-CURRENT: 435/7.23,424/9.6

ABSTRACT:

Kits containing the diagnostic systems and diagnostic systems that rely on bioluminescence for visualizing tissues in situ are provided. The systems include compositions containing conjugates that include a tissue specific, particularly a tumor-specific, targeting agent linked to a targeted agent, a luciferase or luciferin. The systems also include a second composition that contains the remaining components of a bioluminescence generating reaction. Administration of the compositions results production of light by targeted tissues that permits the detection and localization of neoplastic tissue for surgical removal.

RELATED APPLICATIONS

[0001] This application is a continuation of allowed U.S. application Ser. No. 08/908,909, filed Aug. 8, 1997 to Bruce Bryan, entitled "DETECTION AND VISUALIZATION OF NEOPLASTIC TISSUES AND OTHER TISSUES." This application and U.S. application Ser. No. 08/908,909 claim the benefit of priority under 35 U.S.C. .sctn. 119(e) to U.S. provisional application Ser. No. 60/023,374 to Bruce Bryan, filed Aug. 8, 1996, and entitled DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES.

[0002] Subject matter in this application is related to subject matter in allowed U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS", and U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, now U.S. Pat. No. 5,876,995, entitled "BIOLUMINESCENT NOVELTY ITEMS". The subject matter of each of U.S.



application Ser. No. 08/597,274 and U.S. application Ser. No. 08/757,046, and U.S. provisional application Ser. No. 60/023,374 is herein incorporated in its entirety by reference thereto.

----- KWIC -----

#### Cross Reference to Related Applications Paragraph - CRTX:

[0002] Subject matter in this application is related to subject matter in allowed U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS", and U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, now U.S. Pat. No. 5,876,995, entitled "BIOLUMINESCENT NOVELTY ITEMS". The subject matter of each of U.S. application Ser. No. 08/597,274 and U.S. application Ser. No. 08/757,046, and U.S. provisional application Ser. No. 60/023,374 is herein incorporated in its entirety by reference thereto.

#### Summary of Invention Paragraph - BSTX:

[0131] The aequorin system is well known [see, e.g., Tsuji et al. (1986) "Site-specific mutagenesis of the calcium-binding photoprotein aequorin," Proc. Natl. Acad. Sci. USA 83:8107-8111; Prasher et al. (1985) "Cloning and Expression of the cDNA Coding for Aequorin, a Bioluminescent Calcium-Binding Protein," Biochemical and Biophysical Research Communications 126:1259-1268; Prasher et al. (1986) Methods in Enzymology 133:288-297; Prasher, et al. (1987) "Sequence Comparisons of cDNAs Encoding for Aequorin Isotypes," Biochemistry 26:1326-1332; Charbonneau et al. (1985) "Amino Acid Sequence of the Calcium-Dependent Photoprotein Aequorin," Biochemistry 24:6762-6771; Shimomura et al. (1981) "Resistivity to denaturation of the apoprotein of aequorin and reconstitution of the luminescent photoprotein from the partially denatured apoprotein," Biochem. J. 199:825-828; Inouye et al. (1989) J. Biochem. 105:473-477; Inouye et al. (1986) "Expression of Apoequorin Complementary DNA in Escherichia coli," Biochemistry 25:8425-8429; Inouye et al. (1985) "Cloning and sequence analysis of cDNA for the luminescent protein aequorin," Proc. Natl. Acad. Sci. USA 82:3154-3158; Prendergast, et al. (1978) "Chemical and Physical Properties of Aequorin and the Green Fluorescent Protein Isolated from Aequorea forskalea" J. Am. Chem. Soc. 100:3448-3453; European Patent Application 0 540 064 A1; European Patent Application 0 226 979 A2, European Patent Application 0 245 093 A1 and European Patent Application 0 245 093 B1; U.S. Pat. No. 5,093,240; U.S. Pat. No. 5,360,728; U.S. Pat. No. 5,139,937; U.S. Pat. No. 5,422,266; U.S. Pat. No. 5,023,181; U.S. Pat. No. 5,162,227; and SEQ ID Nos. 5-13, which set forth DNA encoding the apoprotein; and a form, described in U.S. Pat. No. 5,162,227, European Patent Application 0 540 064 A1 and Sealite Sciences Technical Report No. 3 (1994), is commercially available from Sealite, Sciences, Bogart, Ga. as AQUALITE.RTM.].

#### Summary of Invention Paragraph - BSTX:

[0235] g. Other Fluorescent Proteins

Summary of Invention Paragraph - BSTX:

[0236] (1) Green and Blue **Fluorescent Proteins**

Summary of Invention Paragraph - BSTX:

[0237] As described herein, blue light is produced using the Renilla luciferase or the Aequorea photoprotein in the presence of  $\text{Ca}^{2+}$  and the coelenterazine luciferin or analog thereof. This light can be converted into a green light if a green **fluorescent protein (GFP)** is added to the reaction. Green **fluorescent proteins**, which have been purified [see, e.g., Prasher et al. (1992) Gene 111 :229-233] and also cloned [see, e.g., International PCT Application No. WO 95/07463, which is based on U.S. application Ser. No. 08/119,678 and U.S. application Ser. No. 08/192,274, which are herein incorporated by reference], are used by cnidarians as energy-transfer acceptors. GFPs fluoresce in vivo upon receiving energy from a luciferase-oxyluciferin excited-state complex or a  $\text{Ca}^{2+}$ -activated photoprotein. The chromophore is modified amino acid residues within the polypeptide. The best characterized GFPs are those of Aequorea and Renilla [see, e.g., Prasher et al. (1992) Gene 111 :229-233; Hart, et al. (1979) Biochemistry 18:2204-2210]. For example, a green **fluorescent protein** [GFP] from Aequorea victoria contains 238 amino acids, absorbs blue light and emits green light. Thus, inclusion of this protein in a composition containing the aequorin photoprotein charged with coelenterazine and oxygen, can, in the presence of calcium, result in the production of green light. Thus, it is contemplated that GFPs may be included in the bioluminescence generating reactions that employ the aequorin or Renilla luciferases or other suitable luciferase in order to enhance or alter color of the resulting bioluminescence.

Summary of Invention Paragraph - BSTX:

[0238] GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source to illuminate neoplasia and specialty tissues, as described herein. Similarly, blue **fluorescent proteins** (BFPs), such as from Vibrio fischeri, Vibrio harveyi or Photobacterium phosphoreum, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue **fluorescent protein** from a yellow-emitting luminous bacterium," Photochem. Photobiol. 55(2): 293-299 (1992); Lee, et al., "Purification of a blue-**fluorescent protein** from the bioluminescent bacterium Photobacterium phosphoreum" Methods Enzymol. (Biolumin. Chemilumin.) 57:226-234 (1978); and Gast, et al. "Separation of a blue **fluorescence protein** from bacterial luciferase" Biochem. Biophys. Res. Commun. 80(1): 14-21 (1978), each incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such **fluorescent proteins** may be used in the methods described herein using a targeting agent conjugate by illuminating the conjugate with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce.

Summary of Invention Paragraph - BSTX:

[0239] Such systems are particularly of interest because no luciferase is needed to activate the photoprotein. These **fluorescent proteins** may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

Summary of Invention Paragraph - BSTX:

[0241] Phycobiliproteins are water soluble **fluorescent proteins** derived from cyanobacteria and eukaryotic algae [see, e.g., Apt et al. (1995) J. Mol. Biol. 238:79-96; Glazer (1982) Ann. Rev. Microbiol. 36:173-198; and Fairchild et al. (1994) J. of Biol. Chem. 269:8686-8694]. These proteins have been used as fluorescent labels in immunoassay [see, Kronick (1986) J. of Immunolog. Meth. 92:1-13], the proteins have been isolated and DNA encoding them is also available [see, e.g., Pilot et al. (1984) Proc. Natl. Acad. Sci. U.S.A. 81:6983-6987; Lui et al. (1993) Plant Physiol 103:293-294; and Houmard et al. (1988) J. Bacteriol. 170:5512-5521; the proteins are commercially available from, for example, ProZyme, Inc., San Leandro, Calif.].

Summary of Invention Paragraph - BSTX:

[0245] As described above for GFPs and BFPs, phycobiliproteins are also activated by visible light of the appropriate wavelength and, thus, may be used in the absence of luciferase and in conjunction with an external light source to illuminate neoplasia and specialty tissues, as described herein. Furthermore, the attachment of phycobiliproteins to solid support matrices is known (e.g., see U.S. Pat. Nos. 4,714,682; 4,767,206; 4,774,189 and 4,867,908). As noted above, these proteins may be used in combination with other **fluorescent proteins** and/or bioluminescence generating systems to produce an array of colors or to provide different colors over time.

Summary of Invention Paragraph - BSTX:

[0248] Thus, when a change in the frequency of emitted light is desired, the phycobiliprotein, or other spectral shifter, such as synthetic fluorochrome, green **fluorescent proteins**, red **fluorescent proteins**, and substrates altered chemically or enzymatically to cause shifts in frequency of emission can be included with the bioluminescent generating components.

Summary of Invention Paragraph - BSTX:

[0374] In alternative embodiments, blue or green light may produced at the target site using a bioluminescence generating system, e.g., using Renilla bioluminescence generating system, and converted to red light by further including a **fluorescent protein**, such as a phycobiliprotein, which converts green light to red light. The concentration of the bioluminescence generating

components necessary to generate red light of sufficient intensity to be detected by the surgical vision device will vary but may be determined empirically by one of skill in the art using methods described herein and those known to those of skill in the art.

#### Summary of Invention Paragraph - BSTX:

[0412] The bioluminescence generating system used to activate the photosensitizing drug will vary depending on the biochemical properties of the drug [e.g., the absorption maxima] and the type of neoplasia to be detected or treated. The selection of the drug and bioluminescence system may be determined empirically based on the teachings herein. In addition, the targeting agent may also include the use of a fluorophore or **fluorescent protein**, e.g., **GFP**, to alter the wavelength of the emitted light to provide a wider range of wavelengths for treatment.

#### Summary of Invention Paragraph - BSTX:

[0414] Presently preferred compounds for use in the methods herein are those that have absorption maxima between 400 and 900 nm wavelengths and particularly preferred bioluminescence generating systems are those that emit wavelengths of light greater than 500 nm, *Aristostomias* and *Malecostus*. In addition, methods using photodynamic therapy with one or more **fluorescent protein** and the bioluminescent generating systems of *Renilla* and the photoprotein aequorin are also preferred.

#### Summary of Invention Paragraph - BSTX:

[0419] Using the coupling methods described herein, a set of microcarriers may be designed containing one or more type of luciferase for the rapid production of a single targeting agent linked to one or more luciferase each having different biochemical properties (i.e., emit light of a different wavelength). In addition, more than one of the bioluminescence generating components may be coupled to the microcarrier. For example, aequorin or *Renilla* luciferase may be coupled, concurrently or successively, with a **GFP**, which absorbs light of one wavelength ( $\lambda_{\text{max}}=480$  nm) and emits light of a different wavelength ( $\lambda_{\text{max}}=509$  nm). Thus, *Renilla* luciferase bound to a microcarrier would emit blue light whereas a microcarrier containing *Renilla* luciferase and **GFP** would emit green light. Alternatively, the coupling a component of the *Aristostomias*, *Pachystomias* or *Malacosteus* bioluminescence generating system to a microcarrier would result in the production of red light.

#### Summary of Invention - Table CWU - BSTL:

1 TABLE OF CONTENTS A. DEFINITIONS B. PREPARATION OF THE CONJUGATES 1. Bioluminescence generating systems a. General description (1) Luciferases (2) Luciferins (3) Activators (4) Reactions b. Ctenophore and coelenterate systems (1) The aequorin system (a) Aequorin and related photoproteins (b)

Luciferin (2) The Renilla system c. Crustacean, particularly Cypridina systems (1) Vargula luciferase (a) Purification from Cypridina (b) Preparation by Recombinant Methods (2) Vargula luciferin (3) Reaction d. Insect bioluminescent systems including fireflies, click beetles, and other insect system (1) Luciferase (2) Luciferin (3) Reaction e. Bacterial systems (1) Luciferases (2) Luciferins (3) Reactions f. Other systems (1) Dinoflagellate bioluminescence generating systems (2) Systems from molluscs, such as Latia and Pholas (3) Earthworms and other annelids (4) Glow worms (5) Marine polychaete worm systems (6) South American railway beetle (7) Fish g. Other **fluorescent proteins** (1) Green and blue **fluorescent proteins** (2) Phycobiliproteins 2. Linkers 3. Targeting Agents C. Formulation and administration of pharmaceutical compositions D. Practice of the reactions in combination with targeting agents E. Kits and compositions 1. Dispensing and Packaging Apparatus for Combination with the Bioluminescent System Components 2. Capsules, pellets, liposomes, endosomes, vacuoles, micronized particles a. Encapsulating vehicles in general b. Encapsulating vehicles -liposomes c. Encapsulating vehicles -gelatin and polymeric vehicles d. Endosomes and vacuoles e. Micronized particles 3. Immobilized systems a. Matrix materials b. Immobilization and activation F. Surgical devices and instruments a. Surgical viewing device b. Surgical imaging instruments G. Photodynamic therapy H. Practice of the methods

#### Detail Description Paragraph - DETX:

[0461] In another embodiment, the location and margins of neoplastic bladder tissue may be defined with greater particularity by detecting the presence of the tumor with targeting agent coupled to the luciferase-bound microparticle. After administration of the target agent conjugate, the bioluminescent reaction is initiated (i.e. by addition of a luciferin and/or any activators). A secondary, **GFP**-bound microparticle is covalently linked to a targeting agent which is directed against nearby surrounding tissue or which preferentially targets identical, non-tumorigenic tissue. The **GFP** conjugate is administered to the patient. Thus, for example, the neoplastic tissue would glow emitting a blue light, e.g., using aequorin or Renilla luciferase-targeting agent conjugate whereas the **GFP**-bound surrounding tissue would absorb the blue light and emit green light thereby providing additional contrast to clearly define the margins of the tissue to be surgically removed.

PGPUB-DOCUMENT-NUMBER: 20020055147

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020055147 A1

TITLE: Human chemokine beta-13

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

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Seibel, George	Saint Davis	PA	US	
Ullrich, Stephen	Rockville	MD	US	
Luscinskas, Francis W.	Milton	MA	US	

APPL-NO: 09/ 908599

DATE FILED: July 20, 2001

RELATED-US-APPL-DATA:

child 09908599 A1 20010720 parent continuation-of 09432768 19991103 US  
ABANDONED child 09432768 19991103 US parent continuation-in-part-of 08986188  
19971205 US ABANDONED child 08986188 19971205 US parent continuation-in-part-of  
08464594 19950605 US PENDING non-provisional-of-provisional 60032432 19961205  
US

US-CL-CURRENT: 435/69.5,435/325 ,530/351 ,536/23.5

ABSTRACT:

The present invention relates to a novel CK.beta.-13 protein which is a member of the chemokine family. In particular, isolated nucleic acid molecules are provided encoding the human CK.beta.-13 protein. CK.beta.-13 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of CKCK.beta.-13 activity. Also provided are diagnostic methods for detecting immune system-related disorders and therapeutic methods for treating immune system-related disorders.

[0001] This application claims benefit of 35 U.S.C. section 120 based on copending U.S. application Ser. No. 08/986,188 filed Dec. 5, 1997, which claimed benefit of 35 U.S.C. section 119(e) based on U.S. Provisional Application Serial No. 60/032,432 filed Dec. 5, 1996. U.S. application Ser. No. 08/986,188 and U.S. Provisional Patent Application Serial No. 60/032,432 are hereby incorporated by reference herein in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0122] Additional preferred polypeptide fragments comprise, or alternatively consist of, the amino acid sequence of residues: M-1 to L-15; A-2 to A-16; R-3 to V-17; L-4 to A-18; Q-5 to L-19; T-6 to Q-20; A-7 to A-21; L-8 to T-22; L-9 to E-23; V-10 to A-24; V-11 to G-25; L-12 to P-26; V-13 to Y-27; L-14 to G-28; L-15 to A-29; A-16 to N-30; V-17 to M-31; A-18 to E-32; L-19 to D-33; Q-20 to S-34; A-21 to V-35; T-22 to C-36; E-23 to C-37; A-24 to R-38; G-25 to D-39; P-26 to Y-40; Y-27 to V-41; G-28 to R-42; A-29 to H-43; N-30 to R-44; M-31 to L-45; E-32 to P-46; D-33 to L-47; S-34 to R-48; V-35 to V-49; C-36 to V-50; C-37 to K-51; R-38 to H-52; D-39 to F-53; Y-40 to Y-54; V-41 to W-55; R-42 to T-56; H-43 to S-57; R-44 to D-58; L-45 to S-59; P-46 to C-60; L-47 to P-61; R-48 to R-62; V-49 to P-63; V-50 to G-64; K-51 to V-65; H-52 to V-66; F-53 to L-67; Y-54 to L-68; W-55 to T-69; T-56 to F-70; S-57 to R-71; D-58 to D-72; S-59 to K-73; C-60 to E-74; P-61 to I-75; R-62 to C-76; P-63 to A-77; G-64 to D-78; V-65 to P-79; V-66 to R-80; L-67 to V-81; L-68 to P-82; T-69 to W-83; F-70 to V-84; R-71 to K-85; D-72 to M-86; K-73 to I-87; E-74 to L-88; I-75 to S-89; C-76 to K-90; A-77 to L-91; D-78 to S-92; and/or P-79 to Q-93 of SEQ ID NO:2. These polypeptide fragments may retain the biological activity of the CK.beta.-13 polypeptides of the invention and may be useful to generate antibodies, as described further below. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Detail Description Paragraph - DETX:

[0417] With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

Detail Description Paragraph - DETX:

[0460] In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

PGPUB-DOCUMENT-NUMBER: 20020025553

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020025553 A1

TITLE: Transforming growth factor alpha HIII

PUBLICATION-DATE: February 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 09/ 726348

DATE FILED: December 1, 2000

RELATED-US-APPL-DATA:

child 09726348 A1 20001201 parent continuation-in-part-of 08778545 19970103 US  
PENDING non-provisional-of-provisional 60011136 19960104 US  
non-provisional-of-provisional 60168387 19991202 US

US-CL-CURRENT: 435/69.1,435/325 ,435/7.1 ,530/399 ,536/23.5

ABSTRACT:

The present invention relates to a novel human protein called Transforming Growth Factor Alpha III, and isolated polynucleotides encoding this protein. Also provided are vectors, host cells, antibodies, and recombinant methods for producing this human protein. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to this novel human protein.

[0001] This application is a continuation-in-part of application Ser. No. 08/778,545, filed Jan. 3, 1997, which claims priority under 35 U.S.C. .sctn.119(e) to application Ser. No. 60/011,136, filed Jan. 4, 1996, each of which is hereby incorporated by reference in its entirety. In addition, this application claims priority under 35 U.S.C. .sctn.119(e) to application Ser. No. 60/168,387, filed Dec. 2, 1999, which is hereby incorporated by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0123] Additional preferred polypeptide fragments comprise, or alternatively



consist of, the amino acid sequence of residues: M-1 to W-15; A-2 to A-16; P-3 to A-17; H-4 to A-18; G-5 to L-19; P-6 to L-20; G-7 to L-21; S-8 to A-22; L-9 to L-23; T-10 to G-24; T-11 to V-25; L-12 to E-26; V-13 to R-27; P-14 to A-28; W-15 to L-29; A-16 to A-30; A-17 to L-31; A-18 to P-32; L-19 to E-33; L-20 to L-34; L-21 to C-35; A-22 to T-36; L-23 to Q-37; G-24 to C-38; V-25 to P-39; E-26 to G-40; R-27 to S-41; A-28 to V-42; L-29 to Q-43; A-30 to N-44; L-31 to L-45; P-32 to S-46; E-33 to K-47; L-34 to V-48; C-35 to A-49; T-36 to F-50; Q-37 to Y-51; C-38 to C-52; P-39 to K-53; G-40 to T-54; S-41 to T-55; V-42 to R-56; Q-43 to E-57; N-44 to L-58; L-45 to M-59; S-46 to L-60; K-47 to H-61; V-48 to A-62; A-49 to R-63; F-50 to C-64; Y-51 to C-65; C-52 to L-66; K-53 to N-67; T-54 to Q-68; T-55 to K-69; R-56 to G-70; E-57 to T-71; L-58 to L-72; M-59 to L-73; L-60 to G-74; H-61 to L-75; A-62 to D-76; R-63 to L-77; C-64 to Q-78; C-65 to N-79; L-66 to C-80; N-67 to S-81; Q-68 to L-82; K-69 to E-83; G-70 to D-84; T-71 to P-85; L-72 to G-86; L-73 to P-87; G-74 to N-88; L-75 to F-89; D-76 to H-90; L-77 to Q-91; Q-78 to A-92; N-79 to H-93; C-80 to T-94; S-81 to T-95; L-82 to V-96; E-83 to I-97; D-84 to L-98; P-85 to D-99; G-86 to L-100; P-87 to Q-101; N-88 to A-102; F-89 to N-103; H-90 to P-104; Q-91 to L-105; A-92 to K-106; H-93 to G-107; T-94 to D-108; T-95 to L-109; V-96 to A-110; L-97 to N-111; I-98 to T-112; D-99 to F-113; L-100 to R-114; Q-101 to G-115; A-102 to F-116; N-103 to T-117; P-104 to Q-118; L-105 to L-119; K-106 to Q-120; G-107 to T-121; D-108 to L-122; L-109 to I-123; A-110 to L-124; N-111 to P-125; T-112 to Q-126; F-113 to H-127; R-114 to V-128; G-115 to N-129; F-116 to C-130; T-117 to P-131; Q-118 to G-132; L-119 to G-133; Q-120 to I-134; T-121 to N-135; L-122 to A-136; I-123 to W-137; L-124 to N-138; P-125 to T-139; Q-126 to I-140; H-127 to T-141; V-128 to S-142; N-129 to Y-143; C-130 to I-144; P-131 to D-145; G-132 to N-146; G-133 to Q-147; I-134 to I-148; N-135 to C-149; A-136 to Q-150; W-137 to G-151; N-138 to Q-152; T-139 to K-153; I-140 to N-154; T-141 to L-155; S-142 to C-156; Y-143 to N-157; I-144 to N-158; D-145 to T-159; N-146 to G-160; Q-147 to D-161; I-148 to P-162; C-149 to E-163; Q-150 to M-164; G-151 to C-165; Q-152 to P-166; K-153 to E-167; N-154 to N-168; L-155 to G-169; C-156 to S-170; N-157 to C-171; N-158 to V-172; T-159 to P-173; G-160 to D-174; D-161 to G-175; P-162 to T-176; E-163 to G-177; M-164 to L-178; C-165 to L-179; P-166 to Q-180; E-167 to C-181; N-168 to V-182; G-169 to C-183; S-170 to A-184; C-171 to D-185; V-172 to G-186; P-173 to F-187; D-174 to H-188; G-175 to G-189; P-176 to Y-190; G-177 to K-191; L-178 to C-192; L-179 to M-193; Q-180 to R-194; C-181 to Q-195; V-182 to G-196; C-183 to S-197; A-184 to F-198; D-185 to S-199; G-186 to L-200; F-187 to L-201; H-188 to M-202; G-189 to F-203; Y-190 to F-204; K-191 to G-205; C-192 to L-206; M-193 to L-207; R-194 to G-208; Q-195 to A-209; G-196 to T-210; S-197 to T-211; F-198 to L-212; S-199 to S-213; L-200 to V-214; L-201 to S-215; M-202 to I-216; F-203 to L-217; F-204 to L-218; G-205 to W-219; I-206 to A-220; L-207 to T-221; G-208 to Q-222; A-209 to R-223; T-210 to R-224; T-211 to K-225; L-212 to A-226; S-213 to K-227; V-214 to T-228; S-215 to S-229 of SEQ ID NO:2. These polypeptide fragments may retain the biological activity of TGF alpha HIII polypeptides of the invention and/or may be useful to generate or screen for antibodies, as described further below. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

#### Detail Description Paragraph - DETX:

[0714] With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter

molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

Detail Description Paragraph - DETX:

[0761] In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

US-PAT-NO: 6518481

DOCUMENT-IDENTIFIER: US 6518481 B1

TITLE: Universal markers of transgenesis

DATE-ISSUED: February 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wimmer; Ernst A.	Bayreuth	N/A	N/A	DE
Berghammer; Andreas J.	Munchen	N/A	N/A	DE
Klingler; Martin	Munchen	N/A	N/A	DE

APPL-NO: 09/ 373129

DATE FILED: August 12, 1999

US-CL-CURRENT: 800/13; 435/320.1 ; 435/455 ; 435/473 ; 536/24.1 ; 800/21

ABSTRACT:

The invention relates to methods, cells and nucleic acids for making transgenic animals. The methods generally comprise introducing into a genome of an animal a genetic construct comprising a transcriptional regulatory element operably linked to a heterologous marker gene encoding a marker, wherein the element drives expression of the marker across genera transgenic in the construct sufficient to visually detect the marker in photoreceptive cells or organs, and selecting for transgenesis by visually detecting the marker in a photoreceptive cell or organ of the animal.

41 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

The subject methods generally comprise (a) introducing into a genome of an animal a genetic construct comprising a transcriptional regulatory element operably linked to a heterologous marker gene encoding a marker, wherein the element drives expression of the marker across genera transgenic in the construct sufficient to visually detect the marker in photoreceptive cells or organs, and (b) selecting for transgenesis by visually detecting the marker in a photoreceptive cell or organ of the animal. In particular embodiments, the construct comprises a vector, such as transposon or retrovirus, particularly a polytropic vector. The construct may integrate into the genome by homologous

or non-homologous recombination. In particular embodiments, the transcriptional regulatory element comprises a binding site selected from a Pax-6 binding site, a Glass binding site, etc., particularly a plurality of P3 sites, and the marker is a **fluorescent protein**, particularly a green **fluorescent protein** or variant thereof.

#### Brief Summary Text - BSTX:

To drive marker expression in a series of diverged organisms requires a promoter which is active in a wide range of species. Furthermore, to avoid problems with low expression and the interference of autofluorescence, a regional specific promoter is preferable over a constitutively active one. A wide variety of regulatory elements may be employed, so long as they meet the requisite functional limitations. These may be natural promoter elements, naturally driving gene expression in photoreceptive cells or organs, elements derived from such natural promoter elements by mutational selection or consensus sequences, synthetic elements derived by iterative selection process, e.g. SELEX procedures, etc. In a particular embodiment, the element comprises a binding site selected from a Pax-6, a Pax-6 like binding site such as a twin-of-eyeless (**TOY**) binding site, a Glass binding site, etc. In more particular embodiments, the element comprises a Pax-6 Paired Domain or Homeodomain binding site, more particularly a P3 site, wherein the P3 site comprises the sequence: TAATYNRATTA (SEQ ID NO:01), wherein Y=C or T; R=G or A; N=any nucleotide (Wilson et al., 1993, Genes Dev 7, 2120-34; Czerny and Busslinger, 1995, Mol Cell Biol 15, 2858-71). Tables 1-6 provide other exemplary transcriptional regulatory element binding sites functional in the subject methods. Pax-6 binding sites are of particular interest due to the evolutionary conserved role Pax-6-homologs play in eye development across different phyla (Callaerts et al., 1997, Annu Rev Neurosci 20, 483-532).

#### Brief Summary Text - BSTX:

The construct includes a marker gene encoding a marker which, when expressed in the transgenic animal, is visually detectable in a photoreceptive cell or organ of the animal. Criteria for marker selection include detectability, physiological and method compatibility, e.g. smaller sized marker genes enable small transposon constructs resulting in high transformation rates. A wide variety of markers may be encoded, including ribozymes or protein enzymes such as galactosidase, luciferase (e.g. Wilson and Hastings, 1998, Annu Rev Cell Dev Biol 14, 197-230), etc., and particularly directly detectable proteins, more particularly **fluorescent proteins**, especially commercially available enhanced **fluorescent proteins** (e.g. EGFP, ECFP and EYFP, Clontech Laboratories, Inc.).

#### Brief Summary Text - BSTX:

Fluorescent proteins may comprise naturally occurring, engineered (i.e., analogs) and/or synthetic sequences. For example, many cnidarians use natural green **fluorescent proteins ("GFPs")** as energy-transfer acceptors in bioluminescence. Natural GFPs have been isolated from numerous animals, including the Pacific Northwest jellyfish, *Aequorea victoria*, the sea pansy,

Renilla reniformis, and Phialidium gregarium; Ward et al., Photochem. Photobiol., 35:803-808 (1982); Levine et al., Comp. Biochem. Physiol., 72B:77-85 (1982). In addition, a variety of Aequorea-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring GFP from Aequorea victoria (Prasher et al., Gene, 111:229-233 (1992); Heim et al., Proc. Natl. Acad. Sci., USA, 91:12501-04 (1994). Particularly useful are GFPs from or which derive from the jellyfish A. victoria (see e.g. U.S. Pat. No. 5,491,084 for applicable such GFPs) and include variants offering a variety of different excitation and emission wavelengths; see e.g. Heim and Tsien, 1996, Current Biology 6, 178-182. Exemplary amino acid variants include F64L, S65T, Y66W, N146I, M153T, V163A and N212K, and combinations thereof. For example, CFP is the GFP of Aequorea victoria with the following additional mutations: F64L, S65T, Y66W, N146I, M153T, V163A, N212K (Miyawaki et al., 1997, Nature 388:882-7), and YFP is the GFP of A. victoria with the following additional mutations: S65G, V68L, S72A, T203Y (Cubitt et al., 1999, Methods Cell Biol 58, 19-30). Accordingly, in preferred embodiments, the marker is a Aequorea or Aequorea-related fluorescent protein, see U.S. Pat. No. 5,912,137 for applicable sequence, scope, definitions and examples.

#### Brief Summary Text - BSTX:

Suitable fluorescent proteins may also derive from other sources, and include the yellow fluorescent protein from Vibrio fischeri strain Y-1 (Baldwin et al., Biochemistry (1990) 29:5509-15) which requires flavins as fluorescent co-factors; Peridinin-chlorophyll, a red fluorescing binding protein from the dinoflagellate Symbiodinium sp. (Morris et al., Plant Mol Biol, (1994) 24:673:77); phycobiliproteins from marine cyanobacteria such as Synechococcus, e.g., phycoerythrin and phycocyanin (Wilbanks et al., J. Biol. Chem. (1993) 268:1226-35), yellow to red fluorescing proteins which require phycobilins as fluorescent co-factors.

#### Detailed Description Text - DETX:

Here we show that an artificial promoter combines the necessary criteria of being hyperactive, regionally restricted and polytropic. We used an artificial promoter containing three Pax6 P3 binding sites in front of a TATA box (3.times.P3). Our P3 site is an idealized (SELEX-derived) paired class homeodomain binding site (Wilson et al., 1993, Genes Dev 7, 2120-34) which in combination with the hsp70 TATA box (-40-+70) is sufficient to regulate photoreceptor cell-specific gene expression in Drosophila (Sheng et al., 1997, Genes Dev. 11, 1122-31). In combination with our marker that encodes an enhanced GFP variant, EGFP (Heim and Tsien, 1996; Tsien, 1998, Annu. Rev. Biochem. 67, 509-44), we can show that this regulatory element does not show any species specificity, and we have used it as a marker for transgenesis in insects of different orders.

#### Claims Text - CLTX:

13. The method according to claim 1, wherein the element comprises a plurality of Pax-6 binding sites and said Pax6 binding sites comprise twin-of-eyeless (**TOY**) binding sites.

Claims Text - CLTX:

15. The method according to claim 1, wherein the marker is a **fluorescent protein**.

Claims Text - CLTX:

16. The method according to claim 1, wherein the marker is a **fluorescent protein and said fluorescent protein** is a green **fluorescent protein**.

Claims Text - CLTX:

36. A method of making a transgenic insect comprising the steps of: (a) introducing into a genome of an insect a polytropic vector functional in nondipteran species and comprising a genetic construct comprising a transcriptional regulatory element operably linked to a heterologous gene encoding a marker, wherein the element comprises a binding site selected from a group consisting of a Pax-6 binding site and a Glass binding site and drives sufficient expression of the marker in insect genera transgenic of the construct to allow visual detection of the marker in photoreceptive cells or organs across said genera, and (b) selecting for transgenesis by visually detecting the marker in a photoreceptive cell or organ of the insect; wherein the marker is a **fluorescent protein**, and wherein the genetic construct is introduced into a vector selected from the group consisting of Himar1, piggyBac, Hermes, hobo, minos and mariner.

Claims Text - CLTX:

37. A polytropic vector functional in nondipteran insect species and comprising a transcriptional regulatory element operably linked to a heterologous gene encoding a marker, wherein the element drives sufficient expression of the marker in insect genera transgenic of the construct to allow visual detection of the marker in photoreceptive cells or organs, wherein the marker is a **fluorescent protein**, and wherein said transcriptional regulatory element and said heterologous gene are introduced into a vector selected from the group consisting of Himar1, piggyBac, Hermes, hobo, minos and mariner.

Other Reference Publication - OREF:

Plautz, J.D. et al. Green **Fluorescent Protein** and its Derivatives as Versatile Markers for Gene Expression in Living *Drosophila Melanogaster*, Plant and Mammalian Cells. *Gene* 173:83-87, 1996.\*

Other Reference Publication - OREF:

Yeh, E. et al. Green **Fluorescent Protein** as a Vital Marker and Reporter of Gene Expression in Drosophila. Proceedings of the National Academy of Science USA 92:7036-7040, Jul. 1995.\*

Other Reference Publication - OREF:

Zhuo, L. et al. Live Astrocytes Visualized by Green **Fluorescent Protein** in Transgenic Mice. Developmental Biology 187:36-42, 1991.\*

US-PAT-NO: 6416960

DOCUMENT-IDENTIFIER: US 6416960 B1

TITLE: Detection and visualization of neoplastic tissues and other tissues

DATE-ISSUED: July 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bryan; Bruce	Beverly Hills	CA	N/A	N/A

APPL-NO: 08/ 908909

DATE FILED: August 8, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application claims the benefit of priority under 35 U.S.C. .sctn.119(e) to U.S. provisional application Ser. No. 60/023,374 to Bruce Bryan, filed Aug. 8, 1996, and entitled DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES.

US-CL-CURRENT: 435/7.23; 424/130.1 ; 424/133.1 ; 424/138.1 ; 424/141.1

ABSTRACT:

Diagnostic systems that rely on bioluminescence for visualizing tissues in situ are provided. The systems are particularly useful for visualizing and detecting neoplastic tissue and specialty tissue during surgical procedures. Kits that provide the components of the systems and methods using the systems for visualizing the tissue are also provided. The systems include compositions containing conjugates that include a tissue specific, particularly a tumor-specific, targeting agent linked to a targeted agent, a luciferase or luciferin. The systems also include a second composition that contains the remaining components of a bioluminescence generating reaction. Administration of the compositions results production of light by targeted tissues that permits the detection and localization of neoplastic tissue for surgical removal. Therapeutic methods in which photosensitizing compounds are administered are also provided.

89 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX:



Subject matter in this application is related to subject matter in U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT **NOVELTY ITEMS**", and U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, entitled "BIOLUMINESCENT **NOVELTY ITEMS**". The subject matter of each of U.S. application Ser. No. 08/597,274 and U.S. application Ser. No. 08/757,046, and U.S. provisional application Ser. No. 60/023,374 is herein incorporated in its entirety by reference thereto.

Brief Summary Text - BSTX:

g. Other **fluorescent proteins**

Brief Summary Text - BSTX:

(1) Green and blue **fluorescent proteins**

Brief Summary Text - BSTX:

The aequorin system is well known [see, e.g., Tsuji et al. (1986) "Site-specific mutagenesis of the calcium-binding photoprotein aequorin," Proc. Natl. Acad. Sci. USA 83:8107-8111; Prasher et al. (1985) "Cloning and Expression of the cDNA Coding for Aequorin, a Bioluminescent Calcium-Binding Protein," Biochemical and Biophysical Research Communications 126:1259-1268; Prasher et al. (1986) Methods in Enzymology 133:288-297; Prasher, et al. (1987) "Sequence Comparisons of cDNAs Encoding for Aequorin Isotypes," Biochemistry 26:1326-1332; Charbonneau et al. (1985) "Amino Acid Sequence of the Calcium-Dependent Photoprotein Aequorin," Biochemistry 24:6762-6771; Shimomura et al. (1981) "Resistivity to denaturation of the apoprotein of aequorin and reconstitution of the luminescent photoprotein from the partially denatured apoprotein," Biochem. J. 199:825-828; Inouye et al. (1989) J. Biochem. 105:473-477; Inouye et al. (1986) "Expression of Apoequorin Complementary DNA in Escherichia coli," Biochemistry 25:8425-8429; Inouye et al. (1985) "Cloning and sequence analysis of cDNA for the luminescent protein aequorin," Proc. Natl. Acad. Sci. USA 82:3154-3158; Prendergast, et al. (1978) "Chemical and Physical Properties of Aequorin and the Green **Fluorescent Protein** Isolated from Aequorea forskalea" J. Am. Chem. Soc. 100:3448-3453; European Patent Application 0 540 064 A1; European Patent Application 0 226 979 A2, European Patent Application 0 245 093 A1 and European Patent Application 0 245 093 B1; U.S. Pat. No. 5,093,240; U.S. Pat. No. 5,360,728; U.S. Pat. No. 5,139,937; U.S. Pat. No. 5,422,266; U.S. Pat. No. 5,023,181; U.S. Pat. No. 5,162,227; and SEQ ID Nos. 5-13, which set forth DNA encoding the apoprotein; and a form, described in U.S. Pat. No. 5,162,227, European Patent Application 0 540 064 A1 and Sealite Sciences Technical Report No. 3 (1994), is commercially available from Sealite, Sciences, Bogart, Ga. as AQUALITE.RTM.].

Brief Summary Text - BSTX:

g. Other **Fluorescent Proteins**

Brief Summary Text - BSTX:

(1) Green and Blue **Fluorescent Proteins**

Brief Summary Text - BSTX:

As described herein, blue light is produced using the Renilla luciferase or the Aequorea photoprotein in the presence of  $\text{Ca}^{2+}$  and the coelenterazine luciferin or analog thereof. This light can be converted into a green light if a green **fluorescent protein (GFP)** is added to the reaction. Green **fluorescent proteins**, which have been purified [see, e.g., Prasher et al. (1992) Gene 111:229-233] and also cloned [see, e.g., International PCT Application No. WO 95/07463, which is based on U.S. application Ser. No. 08/119,678 and U.S. application Ser. No. 08/192,274, which are herein incorporated by reference], are used by cnidarians as energy-transfer acceptors. GFPs fluoresce in vivo upon receiving energy from a luciferase-oxyluciferin excited-state complex or a  $\text{Ca}^{2+}$ -activated photoprotein. The chromophore is modified amino acid residues within the polypeptide. The best characterized GFPs are those of Aequorea and Renilla [see, e.g., Prasher et al. (1992) Gene 111:229-233; Hart, et al. (1979) Biochemistry 18:2204-2210]. For example, a green **fluorescent protein** [GFP] from Aequorea victoria contains 238 amino acids, absorbs blue light and emits green light. Thus, inclusion of this protein in a composition containing the aequorin photoprotein charged with coelenterazine and oxygen, can, in the presence of calcium, result in the production of green light. Thus, it is contemplated that GFPs may be included in the bioluminescence generating reactions that employ the aequorin or Renilla luciferases or other suitable luciferase in order to enhance or alter color of the resulting bioluminescence.

Brief Summary Text - BSTX:

GFPs are activated by blue light to emit green light and thus may be used in the absence of luciferase and in conjunction with an external light source to illuminate neoplasia and specialty tissues, as described herein. Similarly, blue **fluorescent proteins** (BFPs), such as from Vibrio fischeri, Vibrio harveyi or Photobacterium phosphoreum, may be used in conjunction with an external light source of appropriate wavelength to generate blue light. (See for example, Karatani, et al., "A blue **fluorescent protein** from a yellow-emitting luminous bacterium," Photochem. Photobiol. 55(2):293-299 (1992); Lee, et al., "Purification of a blue-**fluorescent protein** from the bioluminescent bacterium Photobacterium phosphoreum" Methods Enzymol. (Biolumin. Chemilumin.) 57: 226-234 (1978); and Gast, et al. "Separation of a blue **fluorescence protein** from bacterial luciferase" Biochem. Biophys. Res. Commun. 80(1):14-21 (1978), each incorporated in its entirety by reference herein.) In particular, GFPs, and/or BFPs or other such **fluorescent proteins** may be used in the methods described herein using a targeting agent conjugate by illuminating the conjugate with light of an appropriate wavelength to cause the **fluorescent proteins** to fluoresce.

#### Brief Summary Text - BSTX:

Such systems are particularly of interest because no luciferase is needed to activate the photoprotein. These **fluorescent proteins** may also be used in addition to bioluminescence generating systems to enhance or create an array of different colors.

#### Brief Summary Text - BSTX:

Phycobiliproteins are water soluble **fluorescent proteins** derived from cyanobacteria and eukaryotic algae [see, e.g., Apt et al. (1995) J. Mol. Biol. 238:79-96; Glazer (1982) Ann. Rev. Microbiol. 36:173-198; and Fairchild et al. (1994) J. of Biol. Chem. 269:8686-8694]. These proteins have been used as fluorescent labels in immunoassay [see, Kronick (1986) J. of Immunolog. Meth. 92:1-13], the proteins have been isolated and DNA encoding them is also available [see, e.g., Pilot et al. (1984) Proc. Natl. Acad. Sci. U.S.A. 81:6983-6987; Lui et al. (1993) Plant Physiol 103:293-294; and Houmard et al. (1988) J. Bacteriol. 170:5512-5521; the proteins are commercially available from, for example, ProZyme, Inc., San Leandro, Calif.].

#### Brief Summary Text - BSTX:

As described above for GFPs and BFPs, phycobiliproteins are also activated by visible light of the appropriate wavelength and, thus, may be used in the absence of luciferase and in conjunction with an external light source to illuminate neoplasia and specialty tissues, as described herein. Furthermore, the attachment of phycobiliproteins to solid support matrices is known (e.g., see U.S. Pat. Nos. 4,714,682; 4,767,206; 4,774,189 and 4,867,908). As noted above, these proteins may be used in combination with other **fluorescent proteins** and/or bioluminescence generating systems to produce an array of colors or to provide different colors over time.

#### Brief Summary Text - BSTX:

Thus, when a change in the frequency of emitted light is desired, the phycobiliprotein, or other spectral shifter, such as synthetic fluorochrome, green **fluorescent proteins**, red **fluorescent proteins**, and substrates altered chemically or enzymatically to cause shifts in frequency of emission can be included with the bioluminescent generating components.

#### Brief Summary Text - BSTX:

In alternative embodiments, blue or green light may be produced at the target site using a bioluminescence generating system, e.g., using Renilla bioluminescence generating system, and converted to red light by further including a **fluorescent protein**, such as a phycobiliprotein, which converts green light to red light. The concentration of the bioluminescence generating components

necessary to generate red light of sufficient intensity to be detected by the surgical vision device will vary but may be determined empirically by one of skill in the art using methods described herein and those known to those of skill in the art.

#### Brief Summary Text - BSTX:

The bioluminescence generating system used to activate the photosensitizing drug will vary depending on the biochemical properties of the drug [e.g., the absorption maxima] and the type of neoplasia to be detected or treated. The selection of the drug and bioluminescence system may be determined empirically based on the teachings herein. In addition, the targeting agent may also include the use of a fluorophore or fluorescent protein, e.g., GFP, to alter the wavelength of the emitted light to provide a wider range of wavelengths for treatment.

#### Brief Summary Text - BSTX:

Presently preferred compounds for use in the methods herein are those that have absorption maxima between 400 and 900 nm wavelengths and particularly preferred bioluminescence generating systems are those that emit wavelengths of light greater than 500 nm, *Aristostomias* and *Malecosteus*. In addition, methods using photodynamic therapy with one or more fluorescent protein and the bioluminescent generating systems of *Renilla* and the photoprotein aequorin are also preferred.

#### Brief Summary Text - BSTX:

Using the coupling methods described herein, a set of microcarriers may be designed containing one or more type of luciferase for the rapid production of a single targeting agent linked to one or more luciferase each having different biochemical properties (i.e., emit light of a different wavelength). In addition, more than one of the bioluminescence generating components may be coupled to the microcarrier. For example, aequorin or *Renilla* luciferase may be coupled, concurrently or successively, with a GFP, which absorbs light of one wavelength ( $\lambda_{\text{max}} = 480 \text{ nm}$ ) and emits light of a different wavelength ( $\lambda_{\text{max}} = 509 \text{ nm}$ ). Thus, *Renilla* luciferase bound to a microcarrier would emit blue light whereas a microcarrier containing *Renilla* luciferase and GFP would emit green light. Alternatively, the coupling a component of the *Aristostomias*, *Pachystomias* or *Malacosteus* bioluminescence generating system to a microcarrier would result in the production of red light.

#### Detailed Description Text - DETX:

In another embodiment, the location and margins of neoplastic bladder tissue may be defined with greater particularity by detecting the presence of the tumor with targeting agent coupled to the luciferase-bound microparticle. After administration of the target agent conjugate, the bioluminescent reaction

is initiated (e.g., by addition of a luciferin and/or any activators). A secondary, **GFP**-bound microparticle is covalently linked to a targeting agent which is directed against nearby surrounding tissue or which preferentially targets identical, non-tumorigenic tissue. The **GFP** conjugate is administered to the patient. Thus, for example, the neoplastic tissue would glow emitting a blue light, e.g., using aequorin or Renilla luciferase-targeting agent conjugate whereas the **GFP**-bound surrounding tissue would absorb the blue light and emit green light thereby providing additional contrast to clearly define the margins of the tissue to be surgically removed.

Claims Text - CLTX:

12. The system of claim 1, wherein the spectral shifter is selected from the group consisting of fluorochrome, green **fluorescent proteins**, red **fluorescent proteins**, and luciferins altered chemically or enzymatically to cause shifts in frequency of emission.

Claims Text - CLTX:

25. The kit of claim 17, wherein the spectral shifter is selected from the group consisting of fluorochrome, green **fluorescent proteins**, red **fluorescent proteins**, and luciferins altered chemically or enzymatically to cause shifts in the frequency of emission.

Claims Text - CLTX:

55. The method of claim 38, wherein the spectral shifter is selected from the group consisting of fluorochrome, green **fluorescent proteins**, red **fluorescent proteins**, and luciferins altered chemically or enzymatically to cause shifts in frequency of emission.

Other Reference Publication - OREF:

Chalfie, Green **fluorescent protein**, Photochemistry and Photobiology, 62(4):651-656 (1995).

Other Reference Publication - OREF:

Delagrave et al., Red-shifted excitation mutants of the green **fluorescent protein**, Bio/Technology 13(2):151-154 (1995).

Other Reference Publication - OREF:

Ehrig et al., Green-**fluorescent protein** mutants with altered fluorescence excitationspectra, FEBS Letters 367:163-166 (1995).

Other Reference Publication - OREF:

Fratamico et al., Construction and characterization of Escherichia coli 0157:H7 strains expressing firefly luciferase and green **fluorescent protein** and their use in survival studies, J of Food Protection 60(10):1167-1173 (1997).

Other Reference Publication - OREF:

Heim et al., Engineering green **fluorescent protein** for improved brightness, longer wavelengths and fluorescence resonance energy transfer, Current Biology 6(2):178-182 (1996).

Other Reference Publication - OREF:

Mitra et al., Fluorescence resonance energy transfer between blue-emitting and red-shifted excitation derivatives of the green **fluorescent protein**, Gene 73(1):13-17 (1996).

Other Reference Publication - OREF:

Romoser et al., Detection in living cells of Ca<sup>2+</sup>-dependent changes in the fluorescence emission of an indicator composed of two green **fluorescent protein** variants linked by a calmodulin-binding sequence, J. of Biolog. Chem. 272(20):13270-13274 (1997).

Other Reference Publication - OREF:

Hart et al., "Renilla reniformis bioluminescence: Luciferase-catalyzed production of nonradiating excited states from luciferin analogues and elucidation of the excited state species involved in energy transfer to Renilla green **fluorescent protein**", (1979) Biochemistry 18:2204-2210 (1979).

Other Reference Publication - OREF:

Hart et al., Renilla reniformis bioluminescence: luciferase-catalyzed production of nonradiating excited states from luciferin analogues and elucidation of the excited state species involved in energy transfer to Renilla green **fluorescent protein**, Biochemistry 18: 2204-2210, (1979).

Other Reference Publication - OREF:

Prasher et al., Primary structure of the Aequorea victoria green-**fluorescent protein**, Gene 111:229-233 (1992).

Other Reference Publication - OREF:

Prendergast et al., Chemical and physical properties of aequorin and the green **fluorescent protein** isolated from Aequorea forsk. ang. lea, Biochemistry 17: 3448-53 (1978).

Other Reference Publication - OREF:

Gast et al., Separation of a blue **fluorescence protein** from bacterial luciferase. Biochem. Biophys. Res. Commun. 80(1): 14-21 (1978).

Other Reference Publication - OREF:

Karatani et al., A blue **fluorescent protein** from a yellow-emitting luminous bacterium, Photochem. Photobiol. 55(2): 293-299 (1992).

US-PAT-NO: 6247995

DOCUMENT-IDENTIFIER: US 6247995 B1

TITLE: Bioluminescent novelty items

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INVENTOR-INFORMATION:

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APPL-NO: 08/ 597274

DATE FILED: February 6, 1996

US-CL-CURRENT: 446/473; 124/74 ; 124/76 ; 222/1 ; 42/54 ; 435/189

ABSTRACT:

Systems and apparatus for generating bioluminescence, and combinations of these systems and apparatus with inanimate articles of manufacture to produce novelty items are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, include, toys, paints, slimy play material, textiles, particularly clothing, bubbles in bubble making toys and other toys that produce bubbles, balloons, personal items, such as bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, foods, such as gelatins, icings and frostings, beverages such as beer, wine, champagne, soft drinks, and ice cubes, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable formulation.

70 Claims, 19 Drawing figures

Exemplary Claim Number: 1,23

Number of Drawing Sheets: 5

----- KWIC -----

TITLE - TI:

Bioluminescent novelty items

Abstract Text - ABTX:



Systems and apparatus for generating bioluminescence, and combinations of these systems and apparatus with inanimate articles of manufacture to produce novelty items are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, include, toys, paints, slimy play material, textiles, particularly clothing, bubbles in bubble making toys and other toys that produce bubbles, balloons, personal items, such as bath powders, body lotions, gels, powders and creams, toothpastes and other dentifrices, soaps, body paints, and bubble bath, foods, such as gelatins, icings and frostings, beverages such as beer, wine, champagne, soft drinks, and ice cubes, fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable formulation.

#### Brief Summary Text - BSTX:

The present invention relates to systems for producing bioluminescent light, and to combinations of the systems with articles of manufacture including toys, textiles, food and beverages, to produce novelty items. By virtue of the combination the novelty items glow or produce or spew a bioluminescent composition. Also, provided are compositions, encapsulated bioluminescent generating reagents, and methods for producing the bioluminescence.

#### Brief Summary Text - BSTX:

Thus, it is an object herein to exploit bioluminescence for use as a recreational product in combination with articles of manufacture to produce novelty items, including toys, personal items, foods, fountains, beverages, coating compositions, such as paints and inks, textiles, including clothing, and other such items. It is also an object herein to provide such combinations and to provide means for producing and using such combinations.

#### Brief Summary Text - BSTX:

Systems and apparatus for generating bioluminescence, and combinations of these systems and apparatus with inanimate articles of manufacture to produce novelty items are provided. These novelty items, which are articles of manufacture, are designed for entertainment, recreation and amusement, include, but are not limited to: toys, particularly squirt guns; finger paints and other paints, slimy play material; textiles, particularly clothing, such as shirts, hats and sports gear suits, threads and yarns; bubbles in bubble making toys and other toys that produce bubbles; balloons; personal items, such as bath powders, body lotions, gels, powders and creams, nail polishes, make-up, toothpastes and other dentifrices, soaps, body paints, and bubble bath; items such as inks, paper; foods, such as gelatins, icings and frostings; and beverages, such as beer, wine, champagne, soft drinks, and ice cubes; fountains, including liquid "fireworks" and other such jets or sprays or aerosols of compositions that are solutions, mixtures, suspensions, powders, pastes, particles or other suitable form.

#### Brief Summary Text - BSTX:

Thus, the novelty items provided herein include but are not limited to: textiles that glow, ink that glows, paints, particularly fingerpaints, that glow, paper products that glow, toys, particularly squirt guns that eject a bioluminescent fluid, dolls and dummies with internal organs or parts that glow; foods and beverages that glow, soapy compositions for blowing bubbles that produce bubbles that glow, bubble bath compositions that produce bubbles that glow, fountains that spew glowing fluid, bioluminescent "fireworks", sparklers, magic-wand toy, and numerous other such items.

#### Brief Summary Text - BSTX:

Bioluminescence is advantageously used combination with the such novelty items because it can be generated using reagents that are nontoxic, noncorrosive and nonstaining. Bioluminescence is also advantageously used because it can be sustained to provide a glow that lasts, if desired, from minutes up to hours.

#### Brief Summary Text - BSTX:

Any article of manufacture that can be combined with a bioluminescence-generating system as provided herein and thereby provide entertainment, recreation amusement, including use of the items for recreation or to attract attention, such as for advertising goods and/or services that are associated with a logo or trademark. Such uses may be in addition to or in conjunction with or in place of the ordinary or normal use of such items. As a result of the combination the items glow or produce, such as in the case of squirt guns and fountains, a glowing fluid or spray of liquid or particles. The novelty in the novelty item derives from its bioluminescence.

#### Brief Summary Text - BSTX:

Methods and compositions for producing bioluminescence in combination with the novelty items are also provided. Micro- and macro-capsular vehicles containing components of bioluminescence generating reactions are provided. The capsular vehicles are capsules, such as liposomes, isolated endosomes, isolated vacuoles, gelatin capsules, and other such delivery vehicles, and the apparatus include vessels, and single chambers, dual chamber and three chamber or more apparatus. These vehicles encapsulate bioluminescent generating system components, and typically contain less than all of the components necessary to generate a bioluminescent reaction. The capsular vehicles include vehicles often used for drug delivery, such as liposomes, and time release capsules; and also capsules made glass, plastic and other such materials.

#### Brief Summary Text - BSTX:

Matrix materials, such as glass, plastics, cotton and other textile material, that contain linked bioluminescence-generating reagents are also provided. For

example, one or more components of the bioluminescence generating system is (are) linked to a matrix material. Matrix materials, such as textiles, glass, a plastic or ceramic surfaces or particles are combined with at least one component of the bioluminescent reagent, particularly the luciferin, luciferase, or, where the components are amenable, the luciferin and luciferase. The component(s) such as the luciferase are linked to the matrix, such as cotton, using methods known to those of skill in the protein synthesis art for linking peptides or proteins to solid substrates [see, e.g., Eichler et al. (1993) Biochemistry 32:11035-11041; Merrifield (1964) Biochemistry 3:1385-1390] Linkage is effected either covalently or non-covalently and can be direct or via linkers. Such methods and linkers are well known to those of skill in the chemical arts. The matrix materials with linked bioluminescent generating system components are contacted with an article of manufacture resulting in a novelty item that, when appropriately treated, such as by spraying on a composition that contains the remaining components of the reactions, glows or produces bioluminescence.

Brief Summary Text - BSTX:

FIG. 4 is a side elevation view, with portions cut away, of a gas powered toy gun with dual chamber detachable fluid reservoir;

Brief Summary Text - BSTX:

FIG. 5 is a top plan view of the toy gun of FIG.4, with portions cut away;

Detailed Description Text - DETX:

a. Single chamber toy guns and other toy weapons that shoot pellets or liquid

Detailed Description Text - DETX:

As used herein, novelty items refer to inanimate articles of manufacture that are intended to provide, even for only a few moments, amusement, entertainment, decoration or recreation. The use for recreation or entertainment may be the items only use or may be in addition to other uses or benefits of the items, such as clothing, including as hats and T-shirts that are modified as described herein by combination with bioluminescence.

Detailed Description Text - DETX:

Novelty items are understood by those of skill in manufacture of such items as well as by the purchasing public and are intended herein to include items such as, toys, including toy guns, dolls, dummies, balloons, bubbles, "fairy dust", such as micronized lyophilized particles, puzzles, and inks and paints, particularly fingerpaints; theatrical vapors when mixed, for example with dry ice or a fog; souvenirs; textiles, particularly clothing, including T-shirts, hats, swimsuits, bathing suit, wet suits, scuba diving suits, surfing suits,

and other water sport or sports attire; foods and beverages, including gelatins, ice cubes, beer, wine, champagne, soft drinks, ice creams, sorbets, ices, frostings, and candy; jewelry, medallions, decorative articles, artificial flowers, articles for displaying names, business tradenames, slogans, trademarks on promotional or other such items, such as T-shirts, hats, paints, wrapping paper, gifts intended to promote business goodwill; personal items, such as body paints, body sprays, bubble baths, make-up, body lotions, dentifrices; fountains; jets or sprays of particles or fluids, including "fireworks", sparklers, and magic-wand toys, and many other such novelty items [see, e.g., U.S. Pat. Nos. 5,435,010, 5,460,022, 5,458,931, 5,435,787, 5,435,010, 5,432,623, 5,421,583, 5,419,558, 5,416,927, 5,413,454, 5,413,332, 5,411,427, 5,410,962, 5,407,691, 5,407,391, 5,405,958, 5,405,206, 5,400,698, 5,399,122, 5,398,972, 5,397,609, 5,396,408, 5,393,580, 5,390,086, 5,389,033, 5,383,684, 5,374,805, 5,368,518, 5,363,984, 5,360,010, 5,353,378, 5,351,931, 5,346,455, 5,341,538, 5,323,492, 5,283,911, 5,222,797, 5,177,812, 5,158,349, 4,924,358, 3,597,877 and many others, which describe types of items are considered novelty items]. Any such inanimate item that is combined with bioluminescence is intended to be encompassed herein.

#### Detailed Description Text - DETX:

Thus, for purposes herein, a novelty item refers to any inanimate article of manufacture that upon combination with bioluminescence provides amusement, entertainment, recreation or enjoyment if only for even a few moments. Addition of the bioluminescence to the article of manufacture does not add to the function of the item, but adds entertainment, amusement or recreational aspects to the item so that the resulting combination is a novelty item. Therefore, the combinations provided herein are novelty items by virtue of the combination an inanimate article of manufacture with bioluminescence.

#### Detailed Description Text - DETX:

As used herein, inanimate means that the articles of manufacture are not live nor formerly living [i.e., dead] items. Thus, the novelty items herein, do not encompass living organisms, such as genetically modified fireflies or genetically engineered plants that express luciferase or other such organisms] that produce bioluminescence.

#### Detailed Description Text - DETX:

In all embodiments, up to all but one component of a bioluminescence generating system will be mixed with or packaged with or otherwise combined with a selected article of manufacture to produce the novelty item. When bioluminescence is desired, the remaining component(s) will be added and light will be produced.

#### Detailed Description Text - DETX:

The aequorin system is well known [see, e.g., Tsuji et al. (1986)

"Site-specific mutagenesis of the calcium-binding photoprotein aequorin," Proc. Natl. Acad. Sci. USA 83:8107-8111; Prasher et al. (1985) "Cloning and Expression of the cDNA Coding for Aequorin, a Bioluminescent Calcium-Binding Protein," Biochemical and Biophysical Research Communications 126:1259-1268; Prasher et al. (1986) Methods in Enzymology 133:288-297; Prasher, et al. (1987) "Sequence Comparisons of cDNAs Encoding for Aequorin Isoforms," Biochemistry 26:1326-1332; Charbonneau et al. (1985) "Amino Acid Sequence of the Calcium-Dependent Photoprotein Aequorin," Biochemistry 24:6762-6771; Shimomura et al. (1981) "Resistivity to denaturation of the apoprotein of aequorin and reconstitution of the luminescent photoprotein from the partially denatured apoprotein," Biochem. J. 199:825-828; Inouye et al. (1989) J. Biochem. 105:473-477; Inouye et al. (1986) "Expression of Apoequorin Complementary DNA in Escherichia coli," Biochemistry 25:8425-8429; Inouye et al. (1985) "Cloning and sequence analysis of cDNA for the luminescent protein aequorin," Proc. Natl. Acad. Sci. USA 82:3154-3158; Prendergast, et al. (1978) "Chemical and Physical Properties of Aequorin and the Green **Fluorescent Protein** Isolated from Aequorea forskalea" J. Am. Chem. Soc. 100:3448-3453; European Patent Application 0 540 064 A1; European Patent Application 0 226 979 A2, European Patent Application 0 245 093 A1 and European Patent Specification 0 245 093 B1; U.S. Pat. No. 5,093,240; U.S. Pat. No. 5,360,728; U.S. Pat. No. 5,139,937; U.S. Pat. No. 5,422,266; U.S. Pat. No. 5,023,181; U.S. Pat. No. 5,162,227; and SEQ ID Nos. 5-13, which set forth DNA encoding the apoprotein; and a form, described in U.S. Pat. No. 5,162,227, European Patent Application 0 540 064 A1 and Sealite Sciences Technical Report No. 3 (1994), is commercially available from Sealite, Sciences, Bogart, Ga. as AQUALITE.RTM.].

#### Detailed Description Text - DETX:

As described herein, blue light is produced using the Aequorea photoprotein in the presence of  $\text{Ca}^{2+}$  or the Renilla luciferase and the coelenterazine luciferin or analog thereof. This light can be converted into a green light if a green **fluorescent protein** is added to the reaction. Green **fluorescent proteins**, which have been purified [see, e.g., Prasher et al. (1992) Gene 111:229-233] and also cloned [see, e.g., International PCT Application No. WO 95/07463, which is based on U.S. application Ser. No. 08/119,678 and U.S. application Ser. No. 08/192,274, which are herein incorporated by reference], is used by cnidarians as energy-transfer acceptors. GFPs fluoresce in vivo upon receiving energy from a luciferase-oxyluciferin excited-state complex or a  $\text{Ca}^{2+}$ -activated photoprotein. The chromophore is modified amino acid residues within the polypeptide. The best characterized GFPs are those of Aequorea and Renilla [see, e.g., Prasher et al. (1992) Gene 111:229-233; Hart, et al. (1979) Biochemistry 18:2204-2210]. For example, a green **fluorescent protein** [GFP] from Aequorea victoria contains 238 amino acids, absorbs blue light and emits green light. Thus, inclusion of this protein in a composition containing the aequorin photoprotein charged with coelenterazine and oxygen, can in the presence of calcium result in the production of green light.

#### Detailed Description Text - DETX:

When used herein, the Renilla luciferase can be packaged, such as in an **toy**, in lyophilized form, encapsulated in a vehicle, either by itself or in combination

with the luciferin substrate. Prior to use the mixture is contacted with an aqueous composition, preferably a phosphate buffered saline pH 7-8; dissolved  $O_2$  will activate the reaction. For use herein, final concentrations of luciferase in the glowing mixture will be on the order of 0.01 to 1 mg/l or more. Concentrations of luciferin will be at least about  $10^{-8}$  M, but 1 to 100 or more orders of magnitude higher to produce a long lasting bioluminescence.

#### Detailed Description Text - DETX:

Lyophilized mixtures, and compositions containing the Renilla luciferase are also provided. The luciferase or mixtures of the luciferase and luciferin may also be encapsulated into a suitable delivery vehicle, such as a liposome, glass particle, capillary tube, drug delivery vehicle, gelatin, time release coating or other such vehicle. Kits containing these mixtures, compositions, or vehicles and also a selected article of manufacture, such as a toy gun, bubble composition, balloon, item of clothing, personal item, are also provided. The luciferase may also be linked to a substrate, such as cotton, polyester, polyester-cotton blends, polypropylene, polyvinyltoluene, polyvinyl propylene, glass, ceramic, or plastics are provided in combination with or as part of an article of manufacture.

#### Detailed Description Text - DETX:

Addition of ATP and luciferin to a reaction that is exhausted produces additional light emission. Thus, the system, once established, is relatively easily maintained. Therefore, it is highly suitable for use herein in embodiments in which a sustained glow is desired or reuse of the item is contemplated. Thus, the components of a firefly system can be packaged with the item of manufacture, such as a toy gun, and then combined with the article before use. For example, the luciferin and ATP can be added to a mild bubble solution that contains luciferase each time the bubbles are used.

#### Detailed Description Text - DETX:

Any toy, vessel or other article of manufacture that is amenable to having a generally translucent covering defining a space for containment of the bioluminescent system components and that is amenable to simple manipulation to permit addition of the final components necessary for the illumination reaction is contemplated.

#### Detailed Description Text - DETX:

Thus, whether the item that will glow or produce a glowing fluid, jet or spray, is a toy, vessel or other article of manufacture, its general design is the same. At least one of the bioluminescent system components is separated from the remaining components. The remaining components are added prior to use. They can be included in the article of manufacture and physically separated from the other components. For example, the physical separation means are

those that are readily removed by the user, to permit mixing, resulting in illumination of the components. For example, an article of manufacture may contain a luciferase and substrate in one compartment and a bioluminescence activator in an adjacent compartment; or alternatively, one compartment may contain the luciferase, and the other the substrate luciferin and dissolved oxygen or other requisite activators. The compartments are separated by a dividing member, such as a membrane, that, upon compression of the article of manufacture, ruptures permitting separated components to mix and to thereby glow. For suitable embodiments, see EXAMPLES, below [see, also, e.g., containers described in U.S. Pat. Nos. 3,539,794 and 5,171,081].

#### Detailed Description Text - DETX:

Other embodiments contemplated herein, include those in which a fluid is ejected as a spray or jet and is rendered bioluminescent prior to ejection from the device, such as a toy or fountain. In general, the methods will involve addition of the bioluminescent system components to the water just prior to ejection thereby causing the ejected spray or jet or stream to glow. Various apparatus for accomplishing this are provided herein. In light of the disclosure herein other apparatus can be adapted for such use. Examples include chambers within a toy that inject the components into a water chamber just prior to ejection of the water, or a clip-on device housing the components, perhaps in pre-measured amounts, which is attached to the toy and manually or automatically engaged to inject the ingredients into a water chamber. Similarly, the water can be introduced into a chamber containing the components and then ejected.

#### Detailed Description Text - DETX:

It will be appreciated, however, that specific applications and configurations of the bioluminescence systems may require specific apparatus. Following are exemplary descriptions of various dispensers and packages contemplated for use herein. These are offered as examples only and are in no way intended as limiting. It is understood that in light of the description herein, other apparatus may be modified or devised, that would be suitable for use to produce bioluminescence in combination with novelty items.

#### Detailed Description Text - DETX:

Certain embodiments of the novelty item combinations provided herein, such as beverage and foods and particles, such as for use as fairy dust or toy guns, fountains of particles and other such applications in require sequestering the components from the environment prior to use or that require particulate form. For example, embodiments in which the bioluminescence generating system is manufactured as part of food or beverage producing glowing beverages or foods require specific packaging considerations. To be amenable to use as an additive to beverages for human consumption, the packaging must be non-toxic, and should be easy to open to provide for contact of the bioluminescence generating system components with the beverage. Examples of suitable packaging for such use include encapsulating the bioluminescent system components in one

or micro- [up to about 100 .mu.m in size] or macroparticles [larger than 100 .mu.M] of material that permits release of the contents, such as by diffusion or by dissolution of the encapsulating material. Liposomes and other encapsulating vehicles [see, e.g., U.S. Pat. No. 4,525,306, which describes encapsulation of compounds in gelatin; U.S. Pat. Nos. 4,021,364, 4,225,581, 4,269,821, 4,322,311, 4,324,683, 4,329,332, 4,525,306, 4,963,368 describe encapsulation of biologically active materials in various polymers] known to those of skill in the art, including those discussed herein and known to those of skill in the art [such as soluble paper, see U.S. Pat. No. 3,859,125]. Likewise, packaging of the system components for addition to food products must address the same considerations. The components may be added to the food substance directly, e.g., by sprinkling the dried and powdered ingredients onto the food, or indirectly, e.g., via addition, to the food, of a capsule containing the ingredients.

#### Detailed Description Text - DETX:

Liposomes are microcapsules [diameters typically on the order of less than 0.1 to 20.mu.m] that contain selected mixtures and can slowly release their contents in a sustained release fashion. Liposomes or other capsule, particularly a time release coating, that dissolve upon exposure to oxygen, air, moisture, visible or ultraviolet [UV] light or a particular pH or temperature [see, e.g., U.S. Pat. No. 4,882,165; Kusumi et al. (1989) Chem. Lett. no.3 433-436; Koch Troels et al. (1990) Bioconjugate Chem. 4:296-304; U.S. Pat. No. 5,482,719; U.S. Pat. No. 5,411,730; U.S. Pat. No. 4,891,043; Straubinger et al. (1983) Cell 32:1069-1079; and Straubinger et al. (1985) FEBS Ltrs. 179:148-154; and Duzgunes et al. in Chapter 11 of the book CELL FUSION, edited by A. E. Sowers; Ellens et al (1984) Biochemistry 23:1532-1538; Yatvin et al. (1987) Methods in Enzymology 149:77-87] may be used for example in the squirt guns or toy machine guns or fairy dust. Liposome formulations for use in baking [see, e.g., U.S. Pat. No. 4,999,208] are available. They release their contents when eaten or heated. Such liposomes may be suitable for incorporation into food products herein or in embodiments in which release of the components by heating is desired.

#### Detailed Description Text - DETX:

The combinations herein are produced by combining a selected novelty item and combining it with a system and apparatus for producing bioluminescence. Selection of the system depends upon factors such as the desired color and duration of the bioluminescence desired as well as the particular item. Selection of the apparatus primarily depends upon the item with which it is combined.

#### Detailed Description Text - DETX:

In other embodiments, the luciferase, such as a Vargula luciferase, is linked to the substrate material, and contacted with a liquid mixture containing the luciferin in an appropriate buffer. Contacting can be effected by spraying or pouring or other suitable manner. The matrix material is incorporated into,



onto or is formed into an article of manufacture, such as clothing or a ceramic, glass, plastic figurine, toy, balloon, flocking agent, such as a Christmas tree flocking agent, or other item. The resulting novelty item can be sold as a kit with a container of the mixture containing the non-linked components, such as in a canister, spray bottle or can, or other suitable format.

#### Detailed Description Text - DETX:

Another embodiment of a dual chamber fluid dispensing apparatus employs a mechanical pump mechanism in its operation. In this embodiment, the dispensing apparatus maintains at least one of the components of the bioluminescence reaction, such as the substrate, luciferase or activator, in separate chambers. A pump mechanism operates to withdraw the contents from each chamber and into a mixing chamber. Within the mixing chamber and upon ejection, the mixed composition is activated, for example by the oxygen in the air or by reaction of the components that were in one chamber, and glows. The pump mechanism may be manually operated, for example by pulling the trigger of a toy squirt gun, or it may be mechanically operated, for example by a motor which operates the pumping mechanism.

#### Detailed Description Text - DETX:

These apparatus may be configured as, for example, a toy gun, toy cannon or other toy weapon, a decorative fountain or volcano or almost any fluid squirting or spouting device. A volcano shaped dispensing apparatus may be used, for example, as a substitute for conventional, similarly shaped fireworks displays.

#### Detailed Description Text - DETX:

Combinations of articles of manufacture and bioluminescence are provided herein. By virtue of the bioluminescence the combinations are novelty items because the bioluminescence provides entertainment, amusement or recreation. Any such combination of an article of manufacture with bioluminescence that produces a novelty item [i.e., provides entertainment, amusement, or recreation] is intended herein. The combination is formed by contacting the article of manufacture or materials in the manufacture with a bioluminescence generating system or an apparatus therefore. The components of the bioluminescence generating system is manufactured as part of the item, coated thereon, impregnated therein, or added after manufacture. Alternatively, the article of manufacture is combined with an apparatus that contains or to which components of the bioluminescence generating system are added, and that produces the bioluminescence.

#### Detailed Description Text - DETX:

The bioluminescent systems provided herein are contemplated for use with various substances to glow the substance. For example, as discussed below, the

bioluminescent system components may be used to produce glowing aqueous mixtures housed in transparent portions of articles of manufacture, thereby illuminating that portion of the article of manufacture. Additionally, the bioluminescent system components may be used to produce glowing food or beverage products, textiles, creams, lotions, gels, soaps, bubbles, papers, powders or water. Following are brief examples of combinations of bioluminescence systems with articles of manufacture and the resulting novelty items contemplated herein.

Detailed Description Text - DETX:

Examples of uses of the bioluminescent systems in toys include illumination of dolls, toy vehicles, hoola hoops, yo-yos, balloons and any other toy amenable to having a generally translucent covering defining a space for containment of the bioluminescent system and addition of the final ingredients necessary for the illumination reaction. Also contemplated herein are toys that eject or spew a fluid. For example, toy or game projectiles are contemplated that contain a luciferase and bioluminescence substrate in an oxygen-free environment. The projectiles rupture upon impact with a hard surface thereby exposing the contents to moisture in the air that contains dissolved oxygen, the bioluminescence activator, and causing reaction.

Detailed Description Text - DETX:

Other toys, games, novelty items, clothes, accessories, foods, beverages, fountains, water dispensing apparatus, soaps, creams, cosmetics and sporting equipment amenable to bioluminescence are further embodiments of the presently disclosed combination. Thus, any article of manufacture or substance capable of modification to allow bioluminescence thereof is contemplated herein.

Detailed Description Text - DETX:

Articles of manufacture that are amenable to use with the bioluminescent systems provided herein are well known [see, e.g., U.S. Pat. Nos. 5,415,151, 5,018,449, 3,539,794, 5,171,081, 4,687,663, 5,038,963, 4,765,510, 4,282,678, 5,366,108, 5,398,827, 5,397,014, 5,219,096, 5,305,919, 5,184,755, 5,029,732, 4,214,674, 4,750,641, 4,676,406], which describe devices useful as toy water guns or vessels for beverages or creams and lotions. To be amenable to use in the embodiments described herein, each may require some modification, such as, for example, addition of a mixing chamber.

Detailed Description Text - DETX:

In light of the disclosure herein, such modification will be apparent. Some of the patents describe other toy devices, training mock weapon devices, dolls, and beverage containers and dentifrice containers [i.e., toothpaste tubes]. In the simplest modification, powdered or capsular vehicles containing bioluminescence generating systems may be added to the water-holding chambers of the toy gun or other water spewing toy. As the powder dissolves or the

vehicle releases its contents, typically luciferin and luciferase, contact with the water in the gun will cause the bioluminescence reaction to occur.

Detailed Description Text - DETX:

As is apparent from the above, toy guns are well known items and materials and specifications for manufacture thereof are also well known [see, the above list and see, also, U.S. Pat. Nos. 5,029,732, and 5,415,151]. Any single chamber squirt gun may be used in combination with bioluminescent systems herein by mixing the components in the gun chamber. Of course the selected system should be one that has sustained illumination. Alternatively, pellets of encapsulated bioluminescent components, such as the aequorin photoprotein or the Renilla luciferase and luciferin, may be added to water in the gun chamber. In the case of the aequorin photoprotein and Renilla luciferase, added tap water may be sufficient. For the Renilla system the pellets may contain the luciferase and luciferin or either. The remaining component will be added to the gun chamber. If pellets are used, the pellets will slowly release their contents thereby providing for a continuous glow.

Detailed Description Text - DETX:

a. Single chamber toy guns and other toy weapons that shoot pellets or liquid

Detailed Description Text - DETX:

Numerous toy guns and other toy weapons that shoot pellets or liquid, in addition to those exemplified herein, are suitable for use in combination with the bioluminescent generating systems herein. The toy weapons may be loaded with a solution containing microspheres of luciferin and/or luciferase, or with lyophilized luciferin/luciferase, or other mixtures as described herein. Suitable toy weapons and devices that shoot jets or sprays of water are described in the following sampling of U.S. Pat. No. 5,462,469 [toy gun that shoots bubbles]; U.S. Pat. No. 5,448,984 [toy gun that shoots balls and water and can be modified to shoot light or temperature sensitive pellets, which should be stored under appropriate conditions or appropriately packaged, that release luciferin/luciferase when exposed to light]; U.S. Pat. Nos. 5,439,139; 5,427,320; 5,419,458; 5,381,928; 5,377,656; 5,373,975; 5,373,833 and 5,373,832 [which describe toy guns that rely upon a pressurizable bladder for release of air pressure to shoot a projectile, which can be modified to shoot projectiles of encapsulated luciferin/luciferase]; U.S. Pat. No. 5,370,278 [which describes liquid from a port mounted to a headband]; U.S. Pat. No. 5,366,108; 5,360,142 [which describes a supply and delivery assembly for use in combination with a pump type water gun or other water propelling device]; U.S. Pat. Nos. 5,346,418; 5,343,850 [which describes a projectile launcher for use in combination with the pellets provided herein]; U.S. Pat. Nos. 5,343,849; 5,339,987 [which describes water guns that have at least one pressurizable air/water storage tank, a pressurizing mechanism, a channel of release for shooting water and a release mechanism]; U.S. Pat. Nos. 5,326,303; 5,322,191; 5,305,919; 5,303,847 [which describes a device worn on a user's hand with sheaths for the tips of the fingers that includes a housing for a water

reservoir, a water pump and electrical motor and a battery pack to be secured to the user's body]; U.S. Pat. Nos. 5,292,032; 5,284,274 [which describes an action to system including a capsule for containing water, which will herein contain components of a bioluminescence generating system, having an orifice and a plunger and a spring loaded mechanism for driving the water from the orifice. The action toy may be configured as a shotgun accepting a plurality of prefilled shell capsules into its breechblock for firing through its barrel, as a missile launcher in which the capsules are mounted to the front of the launcher and the water is ejected directly from the capsule against the target, or as a crossbow with the bow loading the spring-loaded mechanism and a water stream obtained on release of the bow]; U.S. Pat. No. 5,284,272 [which describes a bottle and cap combination for spewing liquid]; U.S. Pat. Nos. 5,256,099; 5,244,153; 5,241,944; 5,238,149; 5,234,129; 5,224,625; 5,213,335; 4,854,480; 5,213,089; 5,184,755; 5,174,477; 5,150,819; 5,141,467; 5,141,462; 5,088,950; 5,071,387 [which describes a figurine-shaped water squirting toy]; U.S. Pat. No. 5,064,095 [which describes a water cannon apparatus]; U.S. Pat. Nos. 5,029,732; 5,004,444; 4,892,228; 4,867,208 [which describes an apparatus for storing and dispensing fluid under pressure]; U.S. Pat. Nos. 4,808,143; 4,784,293; 4,768,681; 4,733,799; 4,615,488 and many others. U.S. Pat. No. 5,415,151 describes a toy gun that launches projectiles that can be adapted for shooting the pellets, such as light sensitive pellets that are degraded upon exposure to light, provided herein.

#### Detailed Description Text - DETX:

Kits containing such soap compositions, with preferably a moderate pH [between 5 and 8] and bioluminescence generating reagents, including luciferase and luciferin are provided herein. These kits can be used with bubble-blowing or producing toy.

#### Detailed Description Text - DETX:

Toys that produce bubbles include bubbles with wand for blowing, bicycles, flying toys, dolls, swords, toy musical instruments, bubble beards, and numerous other toys are well known [see, e.g., U.S. Pat. No. RE 32,973, which describes a toy bubble-blowing lawn mower; U.S. Pat. No. 4,511,497, which describes a non-toxic non-irritating bubble composition for toys, U.S. Pat. Nos. 2,579,714; 5,480,334; 5,041,042; 5,478,267; 5,462,469; 5,419,728; 5,393,256; 5,366,402; 5,348,507; 5,322,464; 5,304,085; 5,269,715; 5,224,893; 5,183,428; 5,181,875; 5,156,564; 5,135,422; 5,080,623; 5,078,636; 4,957,464; 4,955,840; 4,943,255; 4,923,426; 4,867,724; 4,861,303; 4,840,597; 4,808,138; 4,804,346; 4,764,141; 4,700,965; 4,556,392; 4,334,383; 4,292,754; 4,246,717; and many others].

#### Detailed Description Text - DETX:

Dual Chamber Fluid Dispensing Apparatus--Toy Water Gun

#### Detailed Description Text - DETX:

A preferred embodiment of the dual chamber fluid dispensing apparatus is a toy water gun as illustrated in FIGS. 1 through 3. The following description of that preferred embodiment is made with reference to those figures. The toy water gun includes two housings [or chambers] 10, 12 that conveniently may be constructed of injection-molded plastic or other suitable material. The two housings 10, 12 are affixed to one another, such as glued, heat sealed or by other such means, along a median seam 46 to form the body of the water gun. See especially FIGS. 2 and 3.

#### Detailed Description Text - DETX:

As the mixtures leave the cylinders 38, 48, they enter the mixing chamber 20, via the conduit means 44 and second check-valve 34. Luminescence begins either upon mixing of the components or as the mixed composition contacts the air upon expulsion from the toy gun. The mixtures may be powdered, such as those produced by lyophilization, or they may be liquid. If powdered, water can be added prior to use.

#### Detailed Description Text - DETX:

The housings 10, 12 may be filled and refilled through the filling caps 17, 19, respectively, located at the top of each housing. A trigger 14 is attached to a trigger guide 13 which serves to guide the trigger 14 towards two piston assemblies 25. Depression of the trigger 14 activates the two piston assemblies 25. This causes a portion of the composition located in each housing 10, 12 to move through the water gun into a mixing chamber 20 and out a nozzle orifice 22. The preferred embodiment illustrated has a trigger guard 15 which helps prevent accidental discharge of the gun and makes the gun appear more realistic. The sighting aids 21, 23 aid in aiming the toy gun and also serve to make the gun appear realistic.

#### Detailed Description Text - DETX:

#### Dual Chamber Fluid Dispensing Apparatus--Gas-Charged Toy Water Gun

#### Detailed Description Text - DETX:

In contrast to the above-described toy water gun, the gas-charged toy water gun operates using pressurized gas, rather than the piston assembly, to move the bioluminescent mixtures through the system. A preferred embodiment of this device is illustrated in FIGS. 4 and 5. In this embodiment the butt of the water gun 86 houses the two chambers 64, 74 that contain the bioluminescent system components. Further, the butt 86 is detachable and thus readily replaced.

#### Detailed Description Text - DETX:

An alternative embodiment includes a beverage container with two pop-tops, in which one is designed, such as including by having a point at the end, to puncture the bladder and the other can be a typical pop-top that is used for emptying the contents of the can, such as by pouring into a glass or into a person's mouth. Since the novelty of these items resides in the resulting glow in the beverage, the beverage should be poured into a glass, or the container should be transparent or translucent to the bioluminescent light.

Claims Text - CLTX:

an article of manufacture; and a bioluminescence generating system, whereby the combination is a novelty item, wherein the article of manufacture is a toy gun.

Claims Text - CLTX:

b) one or more components of a bioluminescence generating system, whereby the combination is a novelty item, wherein the article of manufacture is a toy gun.

Claims Text - CLTX:

a bioluminescence generating system; wherein the article of manufacture and bioluminescence generating system are packaged in the kit with instructions for combining the article of manufacture with the bioluminescence generating system to produce a novelty item, wherein the article of manufacture is a toy gun.

Claims Text - CLTX:

15. An article of manufacture, comprising packaging material and at least one component of a bioluminescence generating system contained within the packaging material, wherein the concentration of the component(s) is sufficient upon dilution and under appropriate conditions to produce bioluminescence in combination with an article of manufacture, and the packaging material includes a label that indicates that contents are used in combination with an article of manufacture to produce a novelty item, wherein the novelty item is a toy gun.

Claims Text - CLTX:

17. A combination of claim 2 that is a toy gun, comprising:

Claims Text - CLTX:

18. The toy gun of claim 17, wherein the means for moving the contents from the two chambers into the mixing chamber and out the nozzle is a trigger.

Claims Text - CLTX:

19. A combination of a toy gun and a bioluminescence generating system, wherein the toy gun comprises:

Claims Text - CLTX:

20. The toy gun of claim 19, wherein one chamber contains a composition comprising up to all except one component of a bioluminescence generating system, and the other chamber contains a composition comprising the remaining component(s).

Claims Text - CLTX:

21. The toy gun of claim 20, wherein the bioluminescence generating system is selected from among an insect system, a coelenterate system, a ctenophore system, a bacterial system, a mollusk system, and an earthworm system.

Claims Text - CLTX:

22. The toy gun of claim 20, wherein the bioluminescence generating system is selected from among Aequorea, Vargula, Renilla, firefly, and bacterial systems.

Claims Text - CLTX:

23. The combination of a toy gun of and at least one component of a bioluminescence generating system, wherein in the toy gun comprises:

Claims Text - CLTX:

24. A The combination of claim 2, wherein the toy gun comprises:

Claims Text - CLTX:

25. The toy gun of claim 23, wherein the bioluminescence generating system is selected from among an insect system, a coelenterate system, a ctenophore system, a bacterial system, a mollusk system, and an earthworm system.

Claims Text - CLTX:

26. The toy gun of claim 23, wherein the bioluminescence generating system is selected from among Aequorea, Vargula, Renilla, firefly, and bacterial systems.

Claims Text - CLTX:

27. A toy gun, containing a composition comprising one or more components of a bioluminescence generating system.

Claims Text - CLTX:

28. The toy gun of claim 27, wherein the components include a luciferase, a luciferin, or a luciferase and a luciferin.

Claims Text - CLTX:

29. The toy gun of claim 27, wherein the composition is encapsulated in a delivery vehicle.

Claims Text - CLTX:

30. The toy gun of claim 27, wherein the composition is in the form of a powder or paste.

Claims Text - CLTX:

31. A toy water gun, comprising one or more components of a bioluminescence generating system.

Claims Text - CLTX:

32. The toy water gun of claim 31, wherein the toy water gun comprises:

Claims Text - CLTX:

33. The toy water gun of claim 31, wherein the toy water gun comprises:

Claims Text - CLTX:

34. The toy gun of claim 33, further comprising two filler caps, each attached to the butt of the housing and each in communication with a different chamber.

Claims Text - CLTX:

35. The toy gun of claim 33, further comprising two pick-up tubes, each situated within a different and in communication with a different of the conduits.

Claims Text - CLTX:



36. A **toy** gun of claim 33, wherein the two chambers are integral to the butt of the housing and are detachable from the main body of the housing.

Claims Text - CLTX:

37. The **toy** gun of claim 32, further comprising a latch attached to the housing, such that the butt of the housing is releasably secured to the main body of the housing.

Claims Text - CLTX:

the **toy** gun of claim 31; and

Claims Text - CLTX:

61. The **toy** gun of claim 20, wherein a component of the bioluminescence generating system is selected from among brittle star, sea cucumber, cartilaginous, bony fish, ponyfish, flashlight fish, angler fish, midshipman fish, midwater fish marine polychaetes, syllid fireworm, jellyfish, hydroid, sea pansy, earthworm, mollusc, limpet, deep-sea fish, clam, firefly, click beetle, railroad worms and squid bioluminescence generating systems.

Claims Text - CLTX:

62. The **toy** gun of claim 20, wherein a component of the bioluminescence generating system is selected from among Cavarnularia, Ptilosarcus, Styelarula, Acanthoptilum, Parazoanthus, Chiroteuthis, Eucleoteuthis, Onychoteuthis, Watasenia; cuttlefish, Sepiolina; Oplophorus, Sergestes, Gnathophausia, Argyropelecus, Yarella, Diaphus, and Neoscopelus bioluminescence generating systems.

Claims Text - CLTX:

63. The **toy** gun of claim 17, wherein a component of the bioluminescence generating system is selected from among brittle star, sea cucumber, cartilaginous, bony fish, ponyfish, flashlight fish, angler fish, midshipman fish, midwater fish marine polychaetes, syllid fireworm, jellyfish, hydroid, sea pansy, earthworm, mollusc, limpet, deep-sea fish, clam, firefly, click beetle, railroad worms and squid bioluminescence generating systems.

Claims Text - CLTX:

64. The **toy** gun of claim 17, wherein a component of the bioluminescence generating system is selected from among Cavarnularia, Ptilosarcus, Stylatula, Acanthoptilum, Parazoanthus, Chiroteuthis, Eucleoteuthis, Onychoteuthis, Watasenia; cuttlefish, Sepiolina; Oplophorus, Sergestes, Gnathophausia,

Argyrolepecus, Yarella, Diaphus, and Neoscopelus bioluminescence generating systems.

Claims Text - CLTX:

65. The **toy** gun of claim 27, wherein a component of the bioluminescence generating system is selected from among brittle star, sea cucumber, cartilaginous, bony fish, ponyfish, flashlight fish, angler fish, midshipman fish, midwater fish marine polychaetes, syllid fireworm, jellyfish, hydroid, sea pansy, earthworm, mollusc, limpet, deep-sea fish, clam, firefly, click beetle, railroad worms and squid bioluminescence generating systems.

Claims Text - CLTX:

66. The **toy** gun of claim 27, wherein a component of the bioluminescence generating system is selected from among brittle star, sea cucumber, cartilaginous, bony fish, ponyfish, flashlight fish, angler fish, midshipman fish, midwater fish marine polychaetes, syllid fireworm, jellyfish, hydroid, sea pansy, earthworm, mollusc, limpet, deep-sea fish, clam, firefly, click beetle, railroad worms and squid bioluminescence generating systems.

Claims Text - CLTX:

67. The **toy** gun of claim 27, wherein a component of the bioluminescence generating system is selected from among Cavarnularia, Ptilosarcus, Stylatule, Acanthoptilum, Parazoanthus, Chiroteuthis, Eucleoteuthis, Onychoteuthis, Watasenia; cuttlefish, Sepiolina; Oplophorus, Sergestes, Gnathophausia, Argyrolepecus, Yarella, Diaphus, and Neoscopelus bioluminescence generating systems.

Claims Text - CLTX:

69. The **toy** gun of claim 23, wherein the bioluminescence generating system is selected from among an insect system, a coelenterate system, a ctenophore system, a bacterial system, a mollusk system, and an earthworm system.

Claims Text - CLTX:

70. The **toy** gun of claim 23, wherein the bioluminescence generating system is selected from among Aequorea, Vargula, Renilla, firefly, and bacterial systems.

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US-PAT-NO: 5931383

DOCUMENT-IDENTIFIER: US 5931383 A

TITLE: Self-illuminated drinking straw

DATE-ISSUED: August 3, 1999

INVENTOR-INFORMATION:

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APPL-NO: 09/ 017992

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ABSTRACT:

The instant invention provides for illuminated drinking straws which employ chemiluminescent mixtures as lighting sources. The illuminated drinking straw may be used with either hot or cold beverage such as water, fruit juices, soft drinks, coffees and teas, milk products or alcoholic beverages. A new and exciting drinking straw for amusement purposes is intended.

24 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Brief Summary Text - BSTX:

Other non-incandescent, chemical means of producing light which may be advantageously employed include bioluminescent systems, or alternately, chemiluminescent systems based on dioxetanes or other chemiluminescent reagents. Toy and novelty applications which utilizes bioluminescent systems are taught in PCT-WO 97/29319.

Brief Summary Text - BSTX:

The instant invention is directed to the use of a chemiluminescent device in combination with a drinking straw. The unique lighting effects generated from

chemiluminescent lighting devices are enhanced by the inherent optical properties of beverages. Beverage fluid motion, color, clarity and degree of effervescence, if any, all serve to add to the interest of the instant invention. While chemiluminescence has been employed to produce various forms of illuminated drinking vessels and **novelty items** such as "swizzle" sticks, heretofore no device has been produced which utilizes the intrinsically interesting nature of beverage fluid travel in transparent or partially transparent tubes or drinking straws.

#### Brief Summary Text - BSTX:

For example, if the chemiluminescent device is producing a generally green or yellow light and a red beverage is drawn up through the device, the red beverage can filter out certain spectral portions of the chemiluminescent light to produce an apparent color change. Some dyes or coloring agents can be used not only as color filters but as fluorescers. A fluorescent dye functions by converting light of one wavelength to another wavelength. For example, blue light from a chemiluminescent device might be converted to red light by employing an appropriate fluorescer. This red light could be produced even if there was little or no red light emitted by the chemiluminescent device. U.S. Pat. No. 4,379,320 teaches to the use of secondary fluorescers similar to those described above. Of course, if such dyes or fluorescers were to be incorporated into a beverage it is necessary that they be completely safe for consumption. A variety of **fluorescent proteins** exist which may be used in this application, the use of said proteins being taught in PCT-WO 97/29319.